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ABSTRACT:

PROBLEM TO BE SOLVED: To obtain a new peptide, a tripeptide having a specific amino acid sequence, having protease activator activity, being safe even if administered to a living body with no concomitant side effect, and useful as e.g. a digestion promoter, chemical agent for studying protease activity.

SOLUTION: This new tripeptide is expressed by the formula Pro-A-B (A is Phe, Lys, Asn, Tyr or Thr; B is Pro or Trp) and has protease activator activity.

Because this new peptide is decomposed in vivo and metabolized, there is
virtually no risk of causing side effects after it is administered to a living
body, and this new peptide is effective as e.g. a digestion promoter or a
chemical agent for studying protease activity. The protease to be activated by
this new peptide is e.g. plasmin, thrombin, chymotrypsin, trypsin, elastase,
urokinase, papain. This new tripeptide is obtained by synthesis through solid
or liquid phase method followed by purification by column chromatography.

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(54)【発明の名称】　プロテアーゼアクティベーター活性を有するペプチド

(57)【要約】　（修正有）
【解決手段】　Pro-A-B（A=Phe,Lys,Asn,Tyr,Thr;B=Pro,Trpを示す。）のトリペプチドまたはその生理的に認められた塩を有効成分とするプロテアーゼアクティベーター、C-D-Pro（C=Tyr,Glu,Pro;D=Asn,Ser,Arg,Tyrを示す）のトリペプチドまたはその生理的に認められた塩を有効成分とするプロテアーゼアクティベーター、Thioproline-Thr-Trpのトリペプチドまたはその生理的に認められた塩を有効成分とするプロテアーゼアクティベーター、Glu-Argのジペプチドまたはその生理的に認められた塩を有効成分とするプロテアーゼアクティベーター。
【効果】　有効成分がペプチドであり、生体内でアミノ酸に分解され代謝される。そのため、生体に投与した場合に副作用を起こす危険性が極めて少なく、プロテアーゼ活性研究用の薬剤や消化促進剤として有効と考えられる。

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【特許請求の範囲】

【請求項１】　Pro-A-B（ただしA=Phe,Lys,Asn,Tyr,Thr;B=Pro,Trpを示す。）で示されるトリペプチド、またはそれらの生理的に認められた塩を有効成分として含有するプロテアーゼアクティベーター。

【請求項２】　C-D-Pro（ただしC=Tyr,Glu,Pro;D=Asn,Ser,Arg,Tyrを示す）で示されるトリペプチド、またはそれらの生理的に認められた塩を有効成分として含有するプロテアーゼアクティベーター。

【請求項３】　Thioproline-Thr-Trpで示されるトリペプチド、またはそれらの生理的に認められた塩を有効成分として含有するプロテアーゼアクティベーター。

【請求項４】　Glu-Argで示されるジペプチド、またはそれらの生理的に認められた塩を有効成分として含有するプロテアーゼアクティベーター。

【請求項５】　トリペプチドのN末端がアシル化された誘導体、またはそれらの生理的に認められた塩であることを特徴とする特許請求の範囲第１項～４項の何れか１項に記載のプロテアーゼアクティベーター。

【請求項６】　トリペプチドのC末端がアミド化された誘導体、またはそれらの生理的に認められた塩であることを特徴とする特許請求の範囲第１項～４項の何れか１項に記載のプロテアーゼアクティベーター。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明はプロテアーゼアクティベーターとして有用な活性を有するペプチドを含有する組成物に関するものである。

【０００２】

【従来の技術】医薬品としてのトリペプチド及びその誘導体を利用することに関しては、トロンビン活性の阻害剤の研究が知られている（ジャーナル メディカル ケミストリー: vol.37, p.2122,1994年 同:vol.36,p.300,1993年）。また、比較的短いペプチド及びその誘導体の医薬品としての開発研究は、例えば特表平4-502154号公報、特表平4-502306号公報、特表平4-502308号公報、特表平4-502309号公報等にみられる。これらの研究では、そのペプチドのアミノ酸配列にプロリル基が含まれ、配列の長さはアミノ酸の数が５残基以上である。これらのペプチドは腫瘍壊死因子（ＴＮＦ）から誘導されたＴＮＦ改良ペプチドとも言えるものである。このように、短いペプチドを医薬品として利用する研究は幾つかなされていた。短いペプチドは細胞内に取り込まれやすいことが十分考えられ、尚且つそれらは生体内で分解されて無害なアミノ酸となるため、生体への投与に対する副作用はほとんど生じないものと考えられる。故にそれら短いペプチドは癌を始め他の病気の治療薬として将来的に有望な薬剤となりうると考えられる。

【０００３】従来、プロテアーゼの活性化については、炎症の場における反応の一つとして多くの報告がなされてきている。しかしながら、それら報告の多くはプロテアーゼの前駆体が活性化を受けて活性プロテアーゼとして作用することに関するものであり、そのよい例がウロキナーゼやプラスミノーゲンアクティベーターによるプラスミノーゲンの活性化によるプラスミンの生成である。一方、プロテアーゼそのものを活性化する物質の探索研究報告は余り例がなく、一般によく知られたものとしてはキモトリプシンのトリプシンによる活性化等があるが、低分子でしかも小ペプチドのプロテアーゼアクティベーターとなるとこれまでのところ皆無である。

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【０００４】

【発明が解決しようとする課題】本発明の目的は、プロテアーゼアクティベーターとしての活性を有する短いペプチドを提供することにあり、さらに具体的には、本発明の目的は特定のアミノ酸配列を有するトリペプチド、ジペプチド或いはそれらの誘導体を含有するプロテアーゼアクティベーターを提供することにあり、本発明の他の目的は特定のアミノ酸配列を有するトリペプチド、ジペプチド或いはその誘導体の生理的に認められた塩を含有する医薬組成物を提供することにある。

【０００５】

【課題を解決するための手段】本発明者らは、鋭意検討の結果、癌細胞の増殖メカニズムを構成する代謝経路のうち、その重要な代謝経路を決定しているのは蛋白質-蛋白質相互作用であることを突き止め、該相互作用に係る蛋白質の結合ドメインに相当するペプチドは該蛋白質の機能を抑制することが可能であるため、その様な短いペプチドは制癌剤として有用であると考えた。

【０００６】そこで有望なトリペプチドのアミノ酸配列の予測するために種々の癌遺伝子産物、例えば K-Sam、Yes、Ret、Kit、Fms、ErbB、Met、Ros、Sea、Trk、Src、Fgr、Fyn、Lyn、Lck、Hck、Abl、Arg などや、さらにはサークホモロジー（SH）ドメインと呼ばれる配列を有する癌遺伝子産物のコンセンサス配列を検索し、それらの結合配列を予測してそのドメインに相当するトリペプチドないしジペプチドを探索した。その結果、トリペプチドとしてはPro-Phe-Pro、Pro-Lys-Pro、Pro-Asn-Pro、Pro-Tyr-Pro、Tyr-Asp-Pro、Tyr-Ser-Pro、Glu-Arg-Pro、Pro-Tyr-Trp、Thiopro(Thioproline)-Thr-Trp及びこれらのN-アセチル体あるいはC-アミド体等を、ジペプチドとしてはGlu-Arg、D-Glu-Arg、Glu-D-Argを見出した。以上の研究では、蛋白質の結合を阻止することによる薬物探索に目を向けていたが、全く意外なことに合成したペプチドの中にプロテアーゼアクティベーターとしての活性を有するものがあることを見出した。本発明は以上の知見に基づいてなされたものである。

【０００７】即ち、本発明は、

１．　Pro-A-B（ただしA=Phe,Lys,Asn,Tyr,Thr;B=Pro,Trpを示す。）で示されるトリペプチド、またはそれらの生理的に認められた塩を有効成分として含有するプロテ

アーゼアクティベーター、

２．　C-D-Pro（ただしC=Tyr,Glu,Pro;D=Asn,Ser,Arg,Tyrを示す）で示されるトリペプチド、またはそれらの生理的に認められた塩を有効成分として含有するプロテアーゼアクティベーター、

３．　Thioproline-Thr-Trpで示されるトリペプチド、またはそれらの生理的に認められた塩を有効成分として含有するプロテアーゼアクティベーター、

４．　Glu-Argで示されるジペプチド、またはそれらの生理的に認められた塩を有効成分として含有するプロテアーゼアクティベーターを提供するものである。

【０００８】

【発明の実施の形態】本発明に係わるトリペプチドのアミノ酸配列としては例えばPro-Phe-Pro、Pro-Lys-Pro、Pro-Tyr-Pro、Tyr-Ser-Pro、Glu-Arg-Pro、Pro-Tyr-Trpなどである。本発明に係わるジペプチドのアミノ酸配列としては例えばGlu-Argなどである。

【０００９】本発明においては、上記トリペプチド、ジペプチドのN末端のアミノ酸のアシル化されたもの、C末端のアミノ酸のアミド化されたもの、および両端が上記のごとく修飾されたものも利用される。

【００１０】本発明のトリペプチド、ジペプチドのN－アシル化誘導体としてはホルミル基、アセチル基、アリールカルボニル基、芳香族カルボニル基誘導体であり、C－アミド化誘導体としてはアミノ基、アルキルアミノ基、芳香族アミノ基誘導体をあげることができる。

【００１１】本発明のトリペプチド、ジペプチド及びその誘導体の生理的に認められた塩としては塩酸、クエン酸、リン酸、酒石酸、乳酸、酢酸、ギ酸、フマル酸、マレイン酸、コハク酸などがあげられる。

【００１２】本発明に係わるトリペプチド、ジペプチド及びその誘導体の合成は、例えば日本生化学会編集続生化学実験講座2 蛋白質の化学（下）に記載のあるように固相法や液相法のいずれの方法によっても容易に合成することができる。

【００１３】本発明に係わるトリペプチド、ジペプチド及びその誘導体の精製は、シリカゲルカラムクロマトグラフィー、イオン交換カラムクロマトグラフィー等通常の方法で行うことができる。溶出液にも限定はなく水、メタノール、クロロホルム等通常の有機溶媒を用いることができる。

【００１４】本発明に係わるトリペプチド、ジペプチド及びその誘導体の合成を更に詳しく述べれば、まずカルボキシル基をベンジル基（Bzl基）で保護した市販品のアミノ酸とを1-エチル-3-（3-ジメチルアミノプロピル）-カルボジイミド塩酸塩(WSCD HCl)やジシクロヘキシルカルボジイミド(DCCD)等の縮合剤を用いて縮合しジペプチドを得る。本発明のトリペプチド、ジペプチドの縮合反応に用いる溶媒としてはN,N-ジメチルホルムアミド(DMF)、ジメチルスルホキシド(DMSO)、アセトニトリル、1,4-ジオキサン、テトラハイドロフラン(THF)ならびにこれらの混合物が好適である。トリペプチド、ジペプチドの保護基の除去はトリフルオロメタンスルホン酸法（ＴＭＳＦＡ法）やパラジウム／炭素を触媒とした水素接触還元法或は液化フッ化水素（ＨＦ）を用いることができる。水素接触還元法で用いる溶媒としては一般的に用いられているもので問題はない。メタノール（以下MeOHと略記することもある）、酢酸あるいはこれらの混合物が好適である。各合成ステップにおける反応進行や純度のチェックには薄層クロマトグラフィー（ＴＬＣ）の一般的な方法を用いることができる。展開溶媒はクロロホルム－メタノール系、ｎ－ブタノール－酢酸－ピリジン－水（４：１：１：２）系を用い、スポットの確認は臭化水素（ＨＢｒ）－ニンヒドリン法を用いることができる。

【００１５】本発明のプロテアーゼアクティベーターは、以上のようにして得られたトリペプチド、ジペプチド及びその誘導体、またはそれらの生理的に認められた塩を有効成分として含有する以外特に制約はないが、化合物の製剤化に際して通常使用される添加剤を含んでもよい。

【００１６】本発明のプロテアーゼアクティベーターが活性化できるプロテアーゼとしてはプロテアーゼであればよく、その起源、種類を問わないが、好ましくはヒト由来のものであり、プロテアーゼとしては、プラスミン、トロンビン、キモトリプシン、トリプシン、エラスターゼ、ウロキナーゼ、カテプシンＢ、パパイン、ロイシンアミノペプチダーゼが好ましい。

【００１７】以下に実施例により本発明を具体的に説明するが、本発明はこれによって限定されるものではない。

【００１８】

【実施例】

実施例 1　〔Pro-Phe-Pro(PFP)の合成〕

Boc-Phe 2.7g（10ミリモル）、ProOBzl HCl 2.4g（10ミリモル）、1-ヒドロキシベンゾトリアゾール(HOBt) 1.5g（11ミリモル）をDMF 30mlに溶解し、氷冷撹拌下にDMF 10mlに懸濁したWSCD HCl 1.7g（11ミリモル）を滴下した。トリエチルアミン(TEA) 1.8mlを加え、万能ｐＨ試験紙でpH7～8であることを確認した後、室温で終夜撹拌した。析出した沈澱を沪過して除き、沪液を減圧濃縮した後、酢酸エチル(EtOAc) 300mlを加え、4%炭酸水素ナトリウム($NaHCO_3$)の10%食塩(NaCl)水溶液、10%NaCl水溶液、0.4Mクエン酸の10%NaCl水溶液、10%NaCl水溶液の順に洗浄した。EtOAc層に無水硫酸ナトリウム(Na_2SO_4)を加えて脱水後、溶媒を減圧留去し、残渣をデシケーター中で減圧乾固した。この乾固物に4N 塩酸/ジオキサン 40ml、チオアニソール 4mlを加え、氷冷下2時間反応した。これを減圧濃縮した後、ジオキサン 50mlを加え、濃縮する操作を2回繰り返し、同様にジエチルエーテル

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(Et$_2$O)を加え濃縮する操作を2回繰り返した。これを減圧乾固した乾固物に、Z-Pro 1.5g（6.2ミリモル）、HOBt 0.84g（6.8ミリモル）を加え、DMF 30mlに溶解した。氷冷撹拌下、DMF 10mlに懸濁したWSCD HCl 1.2g（6.8ミリモル）を滴下した。TEA 1.6mlでpH7～8に調製した後、室温で1.5時間反応させた。さらに4℃で終夜反応させた後、析出した沈澱を沪過して除き、沪液を減圧濃縮した。EtOAc 300mlを加え、4%NaHCO$_3$の10%NaCl水溶液、10%NaCl水溶液、0.4Mクエン酸の10%NaCl水溶液、10%NaCl水溶液の順に洗浄した。EtOAc層に無水Na$_2$SO$_4$を加えて脱水後、溶媒を減圧留去し、残渣をデシケーター中で減圧乾固した。乾固物を少量のメタノール(MeOH)に溶解し、セファデックス LH-20カラム(内径3cm×長さ55cm)にて分離精製した。同溶媒で溶出し、1フラクションあたり5分（約8ml）で分画した。各フラクションについてＴＬＣ（キーゼルゲル60F$_{254}$ 展開溶媒には、クロロホルム－MeOHの系を用いた。）で確認後、目的物を含む画分を集め減圧乾固した。これをMeOH 20ml、酢酸(AcOH) 20mlの混合液に溶解し、窒素気流下で撹拌した。撹拌を止め、10% パラジウム/カーボン(Pd/C)（51.60% 含水物）2.0gを加え、水素気流を通じた後、常温常圧で激しく撹拌して、反応を開始した。水素の吸収が終了したところで撹拌を止め、窒素ガスで水素ガスを置換した後、触媒を沪別した。沪液を減圧濃縮したものに水を加え、もう一度減圧濃縮した。濃縮物をセファデックス G-10カラム(内径2.6cm×長さ60cm)で分離精製した。水で溶出し、1フラクションあたり5分（約10ml）で分画した。各フラクションの純度をＴＬＣ（展開溶媒は、ｎ－ブタノール：酢酸：ピリジン：水＝４：１：１：２（ＢＡＰＷ系）を用いた。）で確認し(Rf値 0.39)、目的物のスポットを単一に与える画分を集め、減圧乾固した。この乾固物を15mlの水に溶解し、凍結乾燥し、無色アモルファス状目的物 1.0g を得た。ここまでの通算収率は28%であった。元素分析 C$_{19}$H$_{25}$N$_3$O$_4$ MW.359.43 計算値 C 63.49、H 7.01、N 11.69。分析値 C 59.41、H 7.42、N 10.66。

【００１９】実施例 ２　〔Pro-Phe-ProのN-アセチル化体(Ac-PFP)の合成〕

実施例１で得られたPro-Phe-Pro 1.2g（3.3ミリモル）を水10mlに溶解し、これに N-アセチルスクシンイミド(N-ASI) 1.5g（10ミリモル）を加え、1N 水酸化カリウム水溶液でpHを約7に調製後室温で終夜撹拌した。反応液を濃縮後、セファデックス G-10カラム(内径2.6cm×長さ60cm)で分離精製した。各フラクションをＴＬＣで確認し、濃縮乾固するとＴＬＣで単一スポット（展開溶媒BAPW系 Rf=0.56）を与える無色アモルファス状目的物 1.2g（収率90%）が得られた。元素分析 C$_{21}$H$_{27}$N$_3$O$_5$ MW.401.46 計算値 C 62.83、H 6.78、N 10.47。分析値 C 59.69、H 6.50、N 10.97。

【００２０】実施例 ３　〔Pro-Phe-ProのC-アミド化体(PFP NH$_2$)の合成〕

6

Boc-Phe 2.3g（8.8ミリモル）、Pro NH$_2$ 1.0g（8.8ミリモル）、HOBt 1.33g（9.8ミリモル）をDMF 30mlに溶解し、氷冷撹拌下、DMF 10mlに懸濁した WSCD HCl(MW115.24 36.46) 1.7g（8.8ミリモル）を滴下した。TEA 1.7ml加え、万能ｐＨ試験紙でpH7～8であることを確認した後、4℃で終夜反応させた。反応後、実施例１と同様に処理し、Boc-Phe-Pro NH$_2$を無色油状物として得た。これに4N 塩酸/ジオキサン 16ml、チオアニソール 1.5mlを加え、氷冷下1時間撹拌した。実施例１と同様に処理し、得られた残渣にZ-Pro 2.0g（7.9ミリモル）、HOBt 1.1g（8.8ミリモル）を加え、DMF 20mlに溶解した。氷冷撹拌下、DMF10mlに懸濁したWSCD HCl 1.7g（8.7ミリモル）を滴下した。TEA 1.5mlでpH7～8に調製した後、4℃で終夜反応させた。反応後、実施例１と同様の処理を行い、Z-Pro-Phe-Pro NH$_2$の油状物を得た。実施例１と同様にセファデックス LH-20カラムで精製し、ＴＬＣでほぼ単一スポットを得、続いて水素接触還元法により脱保護反応後、セファデックス G-10カラムで精製して、Et$_2$Oから結晶化させ、無色結晶 0.2g（BAPW系 Rf=0.48）を得た。ここまでの通算収率は39%であった。元素分析 C$_{19}$H$_{26}$N$_4$O$_3$ MW.358.44 計算値 C 60.37、H 7.31、N 15.63。分析値 C 60.48、H 7.38、N 14.55。

【００２１】実施例 ４　〔Pro-Phe-ProのN-アセチル化、C-アミド化体(Ac-PFP NH$_2$)の合成〕

実施例３で得られたPFP NH$_2$ 1.0g（2.8ミリモル）に水6mlを加えて溶解し、実施例２と同様に反応・精製することによって目的物 0.9g（収率80%）が得られた。これはＴＬＣで単一スポット（展開溶媒BAPW系 Rf=0.62）を与えた。元素分析C$_{21}$H$_{28}$N$_4$O$_4$ MW.400.25 計算値 C 62.98、H 7.05、N 13.99。分析値 C 58.91、H6.68、N 13.01。

【００２２】実施例 ５　〔Pro-Lys-Pro(PKP)の合成〕

Boc-Lys(Cl-Z) t-Bu NH$_2$ 3.5g（7.1ミリモル）を400mlのEtOAcに溶解し、0.4Mクエン酸の10%NaCl水溶液、10%NaCl水溶液の順に洗浄した。EtOAc層に無水Na$_2$SO$_4$を加えて脱水後、溶媒を減圧留去し、アモルファス状の Boc-Lys(Cl-Z)を得た。これにProOBzl HCl 1.7g（7.1ミリモル）、HOBt 1.0g（7.7ミリモル）とWSCDHCl 1.5g（7.8ミリモル）を加え実施例１と同様に反応・精製し、Boc-Lys(Cl-Z)-ProOBzlを得た。得られた乾固物を実施例１と同様に酸処理してBoc基を除去し、得られた残渣にZ-Pro 1.7g（6.8ミリモル）、HOBt 1.0g（7.5ミリモル）、WSCD HCl 1.5g（7.8ミリモル）を加え実施例１と同様に反応・精製し、油状のZ-Pro-Lyz(Cl-Bzl)-ProOBzlを得た。更にこれをセファデックス LH-20カラム(内径2.6cm×長さ60cm)で精製し、ＴＬＣでほぼ単一のスポットを与えるフラクションを集め、減圧乾固した。これに少量のEtOAcに溶かしたEt$_2$Oを加え、冷却することで結晶化した。結晶を沪取し、真空デシケーター中で減圧乾固

し、無色結晶 2.68gを得た。この結晶をAcOH 36ml、MeOH 24mlに溶解し、実施例１と同様に水素接触還元法で全保護基の除去を行った。セファデックス G-10カラム(内径2.6cm×長さ60cm)で分離精製した。各フラクションをＴＬＣ（展開溶媒BAPW系 Rf=0.009）で確認し、目的のフラクションを集め、濃縮し、さらに凍結乾燥すると無色アモルファス状目的物 1.4gを得た。ここまでの通算収率は63%であった。元素分析 $C_{16}H_{28}N_4O_4$ MW.340.42 計算値 C 56.45、H 8.29、N 16.34。分析値 C 54.06、H8.59、N15.27。

【００２３】実施例 ６　〔Pro-Lys-ProのN-アセチル化体(Ac-PKP)の合成〕

実施例５で得られたPro-Lys-Proのアモルファス状物 1.2g(3.8ミリモル)を水10mlに溶解し、溶液をpH7に調整後、N-ASI 1.6g(11.3 ミリモル）を加え、室温で一晩撹拌した。反応液を濃縮乾固した後、残渣を少量のクロロホルム（$CHCl_3$）に溶解し、$CHCl_3$:MeOH=10:1の溶液で作製したシリカゲル C-200カラム(内径2.2cm×長さ33cm)に層積後、$CHCl_3$:MeOH=10:1の溶液300mlで、そしてMeOHのみで溶出させ、約10mlのフラクションに分画し、各フラクションをＴＬＣで確認して、目的物のスポットを与えるフラクション102から115までを集め、濃縮乾固した。これを少量の水に溶解後、凍結乾燥するとＴＬＣで単一スポット（展開溶媒BAPW系Rf=0.40）を与える無色アモルファス状物 1.25g（76%）が得られた。核磁気共鳴スペクトル(NMR)でシグナルの位置を満足した。1H NMR(DMSO-d_6, δ) 1.82 (3H,s) 2.15(3H,s)。

【００２４】実施例 ７　〔Pro-Lys-Pro NH_2(PKP NH_2)の合成〕

Boc-Lys(Z) 3.5g（9.2ミリモル）、Pro NH_2 1.1g（9.2ミリモル）、HOBt 1.34g（9.9ミリモル）、WSCD HCl 1.95g（10ミリモル）とを実施例１と同様に反応・精製し、目的物 Boc-Lys(Z)-Pro NH_2を無色油状物として得た。実施例１と同様に処理して得られた残渣にZ-Pro 1.5g（6.1ミリモル）、HOBt 0.85g（6.3ミリモル）、WSCD HCl 1.3g（6.8ミリモル）とを加え、実施例１に記載した方法と同様に反応し、Z-Pro-Lys-Pro NH_2を油状物として得、これを実施例１と同様にセファデックス LH-20カラムで精製してＴＬＣでほぼ単一のスポットを与えるものを得た。続いて10%Pd/Cを用いた水素接触還元法により脱保護反応後、少量の水に溶解して、セファデックス G-10カラムで精製して凍結乾燥すると単一スポット（展開溶媒BAPW系 Rf=0.17）を与える目的物 1.1gが得られた。ここまでの通算収率は35%であった。$C_{16}H_{29}N_5O_3$ MW 339.44 計算値 C 56.62、H 8.61、N 20.63。分析値 C 51.56、H 8.83、N 18.63。

【００２５】実施例 ８　〔Pro-Tyr-Pro(PYP)の合成〕

Boc-Tyr(Bzl) 2.0g（5.3ミリモル）、ProOBzl HCl 1.3g（5.38ミリモル）、HOBt 0.81g（ 6.0ミリモル）、WSCD HCl 1.14g（5.9ミリモル）を実施例１と同様に反応させ、目的物 Boc-Tyr(Bzl)-ProOBzlを得た。この乾固物に実施例１と同様に酸処理してBoc基を除去し、得られた残渣に Z-Pro 1.3g（5.1ミリモル）、HOBt 0.76g（5.6ミリモル）、WSCD HCl 1.1g（5.6ミリモル）を滴下し、実施例１と同様に反応させた後、セファデックス LH-20カラム(内径2.6cm×長さ60cm)で分離精製した。減圧濃縮した後、これを少量のEtOAc に溶解し、Et_2Oを加え、冷却することで結晶化した。結晶を沪取し、減圧乾固して2.68gを得た。この結晶を水素接触還元法により脱保護したのち、セファデックス G-10 カラムで精製した。濃縮後、少量のMeOHに溶解し、Et_2Oを添加して結晶化させ、冷却後結晶を沪集し、乾燥すると1.16gが得られた。ここまでの通算収率は55%であった。このものはＴＬＣで単一スポット（展開溶媒BAPW系 Rf=0.32)を与えた。元素分析　$C_{19}H_{25}N_3O_5$ MW375.43 計算値 C 60.79、H 6.71、N 11.19。分析値 C 57.54、H 6.94、N 10.19。

【００２６】実施例 ９　〔Pro-Tyr-ProのN-アセチル化体(Ac-PYP)の合成〕

実施例８で得られたPro-Tyr-Pro 1g（2.6ミリモル）にN-ASI 1.1g（8ミリモル）を加え、実施例２と同様に反応させ、反応液をクロロホルム 20mlで抽出する操作を5回繰り返した。クロロホルム層をNa_2SO_4で脱水後減圧乾固した。これを少量の水に溶解し、凍結乾燥し、ＴＬＣで単一スポット（展開溶媒BAPW系 Rf=0.51）を与える無色アモルファス状物 0.68g（収率61%）を得た。元素分析 $C_{21}H_{27}N_3O_6$ MW417.46 計算値 C 60.42、H 6.52、N 10.07。分析値 C 54.52、H 6.58、N11.04。

【００２７】実施例 １０　〔Pro-Tyr-Pro NH_2(PYP NH_2)の合成〕

Boc-Tyr(Bzl) 3.25g（8.8ミリモル）、Pro NH_2 1.0g（8.8ミリモル）、HOBt 1.2g（8.9ミリモル）にWSCD HCl 1.7g （9.0ミリモル）を加え、実施例１と同様に反応・精製し、Boc-Tyr(Bzl)-Pro NH_2を無色油状物として得た。得られた乾固物を実施例１と同様に酸処理してBoc基を除去し、溶媒を減圧濃縮してEt_2Oを加え結晶を析出させた。この結晶を沪取し、真空デシケーター中で減圧乾固した。乾固した結晶にZ-Pro 2.0g（7.9ミリモル）、HOBt 1.2g（8.9ミリモル）を加え、WSCD HCl 1.7g（9.0ミリモル）を滴下し、実施例１と同様に反応・精製し、油状物を得た。これを少量のメタノールに溶解し、セファデックスLH-20カラム(内径2.6cm×長さ60cm)にかけ、同溶媒で溶出した。目的のフラクションをＴＬＣにて探し、1つに集めた後、減圧乾固し、アモルファス状目的物4.9gを得た。これを水素接触還元法による脱保護反応を行なった後、セファデックス G-10カラムで精製後凍結乾燥し、ＴＬＣで単一スポット（展開溶媒BAPW系Rf=0.42）を与える無色アモルファス状目的物 2.9gを得た。ここまでの通算収率は87%であった。元素分析 $C_{19}H_{26}N_4O_4$ MW 377.44計算値 C 60.95、H 7.60、N14.9

6。分析値 C 56.07、H 7.19、N 13.84。

【００２８】実施例 １１ 〔Pro-Tyr-ProのN-アセチル化、C-アミド化体(Ac-PYP NH_2)の合成〕

実施例１０で得られたPYP NH_2 1.0g（2.6ミリモル）にN-ASI 1.0g（7ミリモル）を加え、実施例２と同様に反応・精製して、ＴＬＣでほぼ単一スポット（展開溶媒BAPW系 Rf=0.61）を与えるフラクションを集め、濃縮し凍結乾燥すると無色アモルファス状目的物 1.05gが得られた。ここまでの収率は96%であった。元素分析 $C_{21}H_{28}N_4O_5$ MW 416.48 計算値 C 60.56、H 6.78、N 13.45。分析値 C 55.11、H 6.01、N 13.09

【００２９】実施例 １２ 〔Tyr-Ser-Pro(YSP)の合成〕

Boc-Ser(Bzl) 2.0g（6.8ミリモル）、ProOBzl HCl 1.6g（6.8ミリモル）、HOBt 1.0g（7.5ミリモル）をDMF 20mlに溶解し、氷冷下撹拌しながら、DMF 10mlに懸濁したWSCD HCl 1.4g（7.3ミリモル）に加え、実施例１に記載した方法と同様に反応させてBoc-Ser(Bzl)-ProOBzlを得た。実施例１に記載した方法と同様に酸処理による脱Boc基反応を行い、得られた残渣にZ-Tyr(Bzl) 2.6g（6.3ミリモル）、HOBt 0.85g（6.3ミリモル）を加え、WSCD HCl 1.3g（6.8ミリモル）を滴下し、実施例１と同様に反応させ、得られた残渣を少量のMeOHに溶かし、セファデックス LH-20カラムで精製した。目的のフラクションをＴＬＣにて探し、集め減圧乾固した。これをEtOAc 50mlに溶解した後、250mlのEt_2Oを加え、冷蔵庫に放置することで結晶化した。結晶を沪取し、減圧乾固した（2.40g)。この結晶を水素接触還元法による脱保護反応を行なった。セファデックス G-10カラムで精製し、凍結乾燥するとＴＬＣで単一スポット（展開溶媒BAPW系 Rf=0.30 ）を与える無色アモルファス状目的物 1.70gが得られた。ここまでの通算収率は68%であった。元素分析 $C_{17}H_{23}N_3O_6$ MW 365.38 計算値 C 56.03、H 6.63、N15.37。 分析値 C 55.91、H 6.81、N 13.82。

【００３０】実施例 １３ 〔Tyr-Ser-ProのN-アセチル化体(Ac-YSP)の合成〕

実施例１２で得られたYSP 1.0g（YSP 2.7ミリモル）にN-ASI 1.1g（8ミリモル）を加え室温で2時間反応させた(pH約8)。反応液を濃縮後、セファデックス G-10カラムで精製し、ＴＬＣで単一のスポット（展開溶媒BAPW系 Rf=0.44）を与える目的物 0.6gが得られた。ここまでの通算収率は54%であった。元素分析 $C_{19}H_{25}N_3O_7$ MW 407.41 計算値 C 56.01、H 6.18、N 10.31。 分析値 C 54.95、H 5.37、N 10.11。

【００３１】実施例 １４ 〔Tyr-Ser-Pro NH_2(YSP NH_2)の合成〕

Boc-Ser(Bzl) 2.6g（8.8ミリモル）、Pro NH_2 1.0g（8.8ミリモル）、HOBt 1.30g（9.7ミリモル）にWSCD HCl 2.0g（10ミリモル）を加え、実施例１と同様に反応させ、Boc-Ser(Bzl)-Pro NH_2を油状物として得た。この油状物を実施例１に記載した方法と同様に酸処理し、溶媒を濃縮した後、Et_2Oを加え、デカンテーションにて沈澱を得た。これを減圧乾固し、Z-Tyr(Bzl)3.2g（7.9ミリモル）、HOBt 1.2g（8.7ミリモル）を加え、DMFに懸濁したWSCDHCl 1.7g（8.8ミリモル）を滴下した。溶媒を濃縮後、少量のEtOAcに溶解し、Et_2Oを加え、冷却することで結晶化した。結晶を沪取し、減圧乾燥すると、3.25g得られた。この結晶を水素接触還元法にて脱保護反応を行い、セファデックス G-10カラムで精製しＴＬＣでほぼ単一のスポット（展開溶媒BAPW系 Rf=0.44）を与える目的物のフラクションを集め濃縮し、凍結乾燥すると、無色アモルファス状物 1.7gが得られた。ここまでの通算収率は53%であった。元素分析 $C_{17}H_{24}N_4O_5$ MW 364.39 計算値 C 56.03、H 6.63、N 15.37。 分析値 C 53.91、H 6.71、N13.82。

【００３２】実施例 １５ 〔Tyr-Ser-ProのN-アセチル化、C-アミド化体(Ac-YSP NH_2)の合成〕

実施例１４で得られたYSP NH_2 0.8g（2.2ミリモル）に、N-ASI 1.0g（7ミリモル）を加え、実施例２に記載した方法と同様に反応させ処理し、乾固物を得た。しかしこれには、縮合剤由来物の混入が推定されたので、少量の水に溶解し、EtOAc 10mlで5回抽出を繰り返し水層を凍結乾燥すると無色アモルファス状物0.87g（収率 95%）が得られた。このものはＴＬＣで単一スポット（展開溶媒BAPW系 Rf=0.56）を与えた。

【００３３】実施例 １６ 〔Glu-Arg-Pro(ERP)の合成〕

Boc-Arg(NO_2) 1/2AcOEt 1/4H_2O 3.7g（10ミリモル）、ProOBzl HCl 2.4g（10ミリモル）、HOBt 1.5g（11ミリモル）にWSCD HCl 2.1g（11.0ミリモル）を加え、実施例１と同様に反応させて処理し、Boc-Arg(NO_2)-ProOBzlを油状物として得た。得られた油状物を実施例１と同様に酸処理した。得られた油状物にZ-Glu(Bzl)1.7g（5.0ミリモル）、HOBt 0.75g（5.7ミリモル）、WSCD HCl 1.1g（5.5ミリモル）を滴下し、実施例１と同様に反応した。得られた残渣を少量のEtOAcに溶解した後、Et_2Oを加え、冷却すると結晶化した。結晶を沪取し、減圧乾固し2.3gの結晶を得た。この結晶を水素接触還元法で全保護器の除去反応を行ない、得られた残渣をセファデックス G-10カラムで精製して、ＴＬＣで単一のスポット（展開溶媒BAPW系 Rf=0.007）を与えるフラクションを集め、濃縮し、凍結乾燥して無色アモルファス状目的物 1.3gを得た。ここまでの通算収率は32%であった。元素分析 $C_{16}H_{28}N_6O_6$ MW 400.44 計算値 C 47.99、H 7.05、N 20.99。分析値 C 45.84、H 7.55、N 19.52。

【００３４】実施例 １７ 〔Glu-Arg-ProのN-アセチル化体(Ac-ERP)の合成〕

実施例１６で得られた ERP 1.1g（2.7ミリモル）に、N-ASI 1.1g（7.1ミリモル）を加え、一晩室温で撹拌した。反応液を濃縮後、セファデックス G-10 カラムで精

製し、各フラクションを集め濃縮し、凍結乾燥して無色アモルファス状目的物 0.80gが得られた。ここまでの通算収率は67%であった。このものはＴＬＣで単一スポット（展開溶媒BAPW系 Rf=0.16）を与えた。元素分析 $C_{18}H_{30}N_6O_7$ MW442.47 計算値 C48.86、H6.83、N18.99。分析値 C45.89、H7.03、N18.07。

【００３５】実施例 １８ 〔Glu-Arg-ProのC-アミド化体(ERP NH_2)の合成〕

Boc-Arg(NO_2) 1/2AcOEt 1/4H_2O 3.7g（10.0ミリモル）、ProOBzl HCl 2.4g（10ミリモル）、HOBt 1.49g（11ミリモル）にWSCD HCl 2.1g（11ミリモル）を加え、実施例１と同様に反応し、EtOAcの代わりにクロロホルムを用いて処理した。クロロホルム層を減圧濃縮した残渣に、EtOAcを加えると白濁した。さらにEt_2Oを加えて4℃で一晩放置して得られたガム状沈澱物をデカンテーションして得、減圧乾固した。得られた乾固物を実施例１と同様に酸処理してBoc基を除去し、反応中生じた沈澱物をデカンテーションで得、減圧乾固した。これにZ-Glu(Bzl) 1.7g（5.0ミリモル）、HOBt 0.75g（5.5ミリモル）を加えWSCD HCl 1.1g（5.7ミリモル）を滴下し、実施例１と同様に反応し、処理をした後、カラムで精製して無色油状物を得た。この油状物を水素接触還元法で全保護基の除去反応を行なった後、セファデックス G-10カラムで精製して、ＴＬＣでほぼ単一スポット（展開溶媒BAPW系 Rf=0.21）のフラクションを集め結晶性残渣を得た。これに冷MeOHを加えて結晶をほぐし沪集すると0.2gが得られた。ここまでの通算収率は6%であった。元素分析 $C_{16}H_{29}N_7O_5$ MW399.45 計算値 C 48.11、H 7.32、N 24.55。分析値 C 46.61、H 6.92、N22.34。

【００３６】実施例 １９ 〔Pro-Thr-Trp(PTW)の合成〕

Boc-ThrOSu 3.7g（12ミリモル）、TrpOBzl HCl 3.8g（12ミリモル）のDMF溶液に、氷冷撹拌下、TEAを加えて、pH7～8であることを確認後、一晩反応させた。実施例１と同様に後処理して、Boc-Thr-TrpOBzlを油状物として得た。続いて実施例１と同様に酸処理して、Boc基を除去し得られた残渣にZ-Pro 2.8g（11ミリモル）を加え、DCCD 2.5g（12ミリモル）のDMF 10ml溶液を滴下し、4℃で一晩反応させた。沈澱物を沪別後、沪液を減圧濃縮し、実施例１と同様に処理してアモルファス状物 4.21gが得られた。このうち2gを水素接触還元法で保護基を除去し、得られた残渣を少量の水に溶解し、セファデックス G-10カラムで精製して、ＴＬＣでほぼ単一スポット（展開溶媒BAPW系 Rf=0.33）を与えるフラクションを集め減圧乾固した。残渣に少量のMeOHを加えて、結晶化させ、冷却後、結晶を沪取し乾燥し1.40gを得た。ここまでの通算収率は30%であった。元素分析 $C_{20}H_{25}N_4O_5$ MW 402.22 計算値 C 59.69、H 6.51、N 13.91。分析値 C 55.90、H 6.41、N 12.75。

【００３７】実施例 ２０ 〔Pro-Thr-Trp NH_2(PTW NH_2)の合成〕

Boc-ThrOSu 6.0g（19ミリモル）をDMF 50mlに溶解し、Trp NH_2塩酸塩 4.6g（19ミリモル）とHOBt 2.9g（21ミリモル）とを粉末のまま加え撹拌溶解した。氷冷撹拌下にTEAを添加し、pH7～8であることを確認後 WSCD HCl 4.1g（21ミリモル）の DMF 10ml溶液を加え、4℃で一晩反応させた。沈殿物を沪去し、沪液を減圧濃縮後 EtOAc 500mlを加えて、実施例１と同様に処理することによって目的物Boc-Thr-Trp NH_2を無色油状物として8.3g(87%)を得た。これを実施例１と同様に反応させ処理して油状物 7.2g（16ミリモル,98%）を得た。続いてこれに Z-Pro 4.1g（16ミリモル）、HOBt 2.5g(18ミリモル）、WSCD HCl 3.5g（18ミリモル）を加え、実施例１と同様に反応させ処理して、Z-Pro-Thr-Trp NH_2をアモルファス状物として7.3g(70%)を得た。このアモルファス状物2.5gに10% Pd/C 1gを加えて実施例１と同様に反応・処理後、脱保護反応を行った。得られたものを少量の水に溶解してセファデックス G-10カラムで精製し、凍結乾燥すると、単一スポット（展開溶媒BAPW系 Rf=0.47）からなる目的物 1.0gが得られた。元素分析 $C_{20}H_{27}N_5O_4$ MW.401.33 計算値 C 59.80、H 6.78、N 17.46。 分析値 C 57.63、H 6.94、N 16.27。

【００３８】実施例 ２１ 〔Pro-Thr-Trp NH_2のN-アセチル化体(Ac-PTW NH_2)の合成〕

実施例２０で得られたPTW NH_2 0.95g（2.4ミリモル）にN-ASI 0.9g（6ミリモル）を加え、室温で一晩反応させたのち、減圧下に濃縮乾固した。少量のクロロホルムに溶かし、クロロホルム：メタノール＝３０：１の混液で作製したシリカゲル C-200（和光純薬製）カラム(内径2.6cm×長さ45cm)に層積し、クロロホルム：メタノール＝３０：１混液 400ml、１０：１混液 300ml、メタノールのみ500mlで溶出させ、各フラクション（約10ml）をＴＬＣでチェック後、フラクション72から75までを集めた。濃縮乾固後、残渣を少量の水に溶解し凍結乾燥すると無色アモルファス状物 0.8gが得られた。ここまでの通算収率は75%であた。このものはＴＬＣで単一スポット(展開溶媒BAPW系 Rf=0.64)を与えた。元素分析 $C_{22}H_{29}N_5O_3$ MW443.50 計算値 C 59.58、H 6.59、N 15.79。分析値 C 56.98、H 6.54、N 15.34。

【００３９】実施例 ２２ 〔thioPro-Thr-Trp(P'TW)の合成〕

Boc-ThrOSu 3.7g（12ミリモル），TrpOBzl HCl 3.8g（12ミリモル）のDMF溶液に、氷冷撹拌下、TEAを加えて、pH7-8であることを確認後、一晩反応させた。実施例１と同様に後処理して、Boc-Thr-TrpOBzlを油状物として得た。続いて実施例１と同様に酸処理してBoc基を除去し、得られた残渣にBoc-thioPro2.8g（12ミリモル）を加え、HOBt 1.784g（13.2ミリモル）、WSCD HCl 2.53g（13.2ミリモル）のDMF 10ml溶液を滴下し、４℃で一晩反応させた。沈澱物を沪別後、沪液を減圧濃縮し、実施

例1と同様に処理してアモルファス状物 6.3gが得られた。カップリングにより得られたBoc-thioPro-Thr-TrpOBzl 6.3gのHFによる保護基の切断は以下の通りに行った。Boc-thioPro-Thr-TrpOBzl 6.3gを10等分して、それぞれHFにより切断した。即ち、Boc-thioPro-Thr-TrpOBzlを反応容器に採り、チオアニソール 11mlを加えて室温に1時間置いた。反応容器をドライアイスーアセトン浴で冷却下、HF 100mlを導入、0℃で1時間撹拌しながら反応させた。水流アスピレーターでHFを減圧除去し、更に真空ポンプにて乾燥させた。得られた残渣を10%酢酸水溶液100mlにて溶解し、50mlのジエチルエーテルで2回洗浄し、残存するチオアニソールを除去した。酢酸水溶液画分を中和して、濃縮乾固させたもの3.2gを少量のクロロホルムーメタノール混液（5：1）に溶解し、シリカゲルカラムクロマトグラフィーにより精製し、目的物を得た。収量0.6g。TLC（BAPW系Rf＝0.62）は単一のスポットを与えた。元素分析　$C_{19}H_{26}N_4O_5S$ MW 420.53 計算値 C 54.22,H 5.99, N 13.35。分析値 C 55.90,H 6.41,N 12.75。

【００４０】実施例　２３　〔D-Pro-Thr-Trp NH_2(D-PTW NH_2: ProはD体)の合成〕

Boc-ThrOSu 3.7g（12ミリモル），TrpOBzl HCl 3.8g（12ミリモル）のDMF溶液に、氷冷撹拌下、TEAを加えて、pH7-8であることを確認後、一晩反応させた。実施例1と同様に後処理して、Boc-Thr-TrpOBzlを油状物として得た。続いて実施例1と同様に酸処理して、Boc基を除去し、得られた残渣にZ-D-Pro2.8g（11ミリモル）を加え、DCCD 2.5g（12ミリモル）のDMF 10ml溶液を滴下し、4℃で一晩反応させた。沈澱物を沪別後、沪液を減圧濃縮し、実施例1と同様に処理してアモルファス状物 4.21gが得られた。このうち　2gを接触還元法で保護基を除去し、得られた残さを少量の水に溶解し、セファデックスG-10カラムで精製して、TLCでほぼ単一スポットを与えるフラクションを集め減圧乾固した。残渣に少量のMeOHを加えて、結晶化させ、冷却後、結晶を沪取し乾燥し1.40g（通算収率30%）を得た（BAPW系 Rf＝0.33）。元素分析 $C_{20}H_{25}N_4O_5$ MW 402.22 計算値 C 59.69, H 6.51,N 13.91。分析値 C 55.90,H 6.41,N 12.75。

【００４１】実施例　２４　〔Glu-D-Arg(E-D-R: ArgはD体)の合成〕

Z-Glu(Bzl)OH 3.4g（10ミリモル）、D-Arg(NO_2)OBzl Tos 4.82g（10ミリモル）、HOBt 1.5g（11ミリモル）をDMF 100mlに溶解、氷冷下、WSCD HCl 2.1g（11ミリモル）を加え、実施例1と同様に反応させ、後処理を行って、Z-Glu(Bzl)-D-Arg(NO_2)OBzlの油状物5.3g（8ミリモル）を得た。この油状物を接触還元法にて脱保護反応を行い、セファデックスG-10カラムで精製し、TLCでほぼ単一のスポットを与える目的物のフラクションを集め、濃縮、凍結乾燥を行った。無色アモルファス状物として1.82g（6ミリモル）を得た（TLC: BAPW系　Rf＝0.5

8）。元素分析　$C_{11}H_{21}N_5O_5$、MW 303.33 計算値 C 43.52, H 6.92, N 23.14。　分析値 C 42.98, H 6.88, N 22.76

【００４２】試験例１　プロテアーゼ活性化作用

プロテアーゼ活性化作用は以下に示す方法により測定した。即ち、下記の各種プロテアーゼの各溶液を別個に調製し、それぞれの溶液に以下に列挙したペプチド（A～Q）を加えて37℃、10分間インキュベートし、1-クロル酢酸ナトリウム、酢酸、酢酸ナトリウムの混合水溶液で反応を停止させた後、340、450nmの2波長による吸光度を測定し、各々のペプチド間の相対強度（%）として図1（図1）、図2（図2）、図3（図4）及び図4（図4）に示した。

プラスミン溶液：プラスミン 20μgを10μM BocVal-Leu-LysMCAを含有するリン酸緩衝液に溶解した。

トロンビン溶液：トロンビン 1.1ngを150mM NaCl、2.5mM $CaCl_2$、10μM BocVal-Pro-ArgMCAを含有する50mMトリス塩酸緩衝液（pH7.5）

キモトリプシン溶液：キモトリプシン 25ngを10μM Suc Leu-Leu-Val-TyrMCAを含有するリン酸緩衝液に溶解した。

トリプシン溶液：トリプシン 100ngを1mM EDTA、10μM BocPhe-Ser-ArgMCAを含有するリン酸緩衝液に溶解した。

エラスターゼ溶液：エラスターゼ 15μgを1mM EDTA、10μM SucAlaProAlaMCAを含有する50mMトリス塩酸溶液（ｐＨ８．８）に溶解した。

ウロキナーゼ溶液：ウロキナーゼ 1mUを1mM EDTA、10μM Pyr-Gly-ArgMCAを含有するリン酸緩衝液に溶解した。

カテプシンB溶液：カテプシンB 350μUを2mM EDTA、2mM DTT、10μM ZArg-ArgMCAを含有する50mM MES緩衝液（pH6.0）に溶解した。

パパイン溶液：パパイン 2.5ngを2mM EDTA、2mM DTT、10μM ZPhe-ArgMCAを含有する50mM MES緩衝液（ｐＨ５．５）に溶解した。

ロイシンアミノペプチダーゼ溶液：ロイシンアミノペプチダーゼ 100μUを5mM $MgCl_2$、10μM LeuMCAを含有する50mM トリス塩酸緩衝液（pH7.4）に溶解した。

【００４３】

【発明の効果】本発明により、トリペプチド、ジペプチド及びそれらの誘導体を含有する全く新規なプロテアーゼアクティベーターが提供される。本発明のプロテアーゼアクティベーターはその有効成分がペプチドであり、生体内でアミノ酸に分解され代謝される。そのため、生体に投与した場合に副作用を起こす危険性が極めて少ない。よって、プロテアーゼ活性研究用の薬剤或いは消化促進剤として有効であることが考えられる。

【図面の簡単な説明】

【図１】　合成ペプチドの各種プロテアーゼ活性化作用を表す図である。

【図2】　合成ペプチドの各種プロテアーゼ活性化作用を表す図である。

【図3】　合成ペプチドの各種プロテアーゼ活性化作用を表す図である。

【図4】　合成ペプチドの各種プロテアーゼ活性化作用を表す図である。

【符号の説明】

図中のA～Qは以下のペプチドを示す。

A：ThioPro-Thr-Trp

B：D-Pro-Thr-Trp

C：Glu-Arg-Pro

D：Glu-Arg-Pro-アミド

E：Glu-Arg

F：D-Glu-Arg

G：Glu-D-Arg

H：Pro-Phe-Pro

I：Pro-Phe-Pro-アミド

J：Pro-Thr-Trp-塩酸塩

K：Pro-Lys-Pro-アミド

L：Tyr-Ser-Pro

M：N-アセチル-Pro-Phe-Pro-アミド

N：Pro-Tyr-Pro-アミド

O：N-アセチル-Pro-Tyr-Pro-アミド

P：Pro-Tyr-Pro-スルホン酸塩

Q：Tyr-Ser-Pro-アミド

【図3】

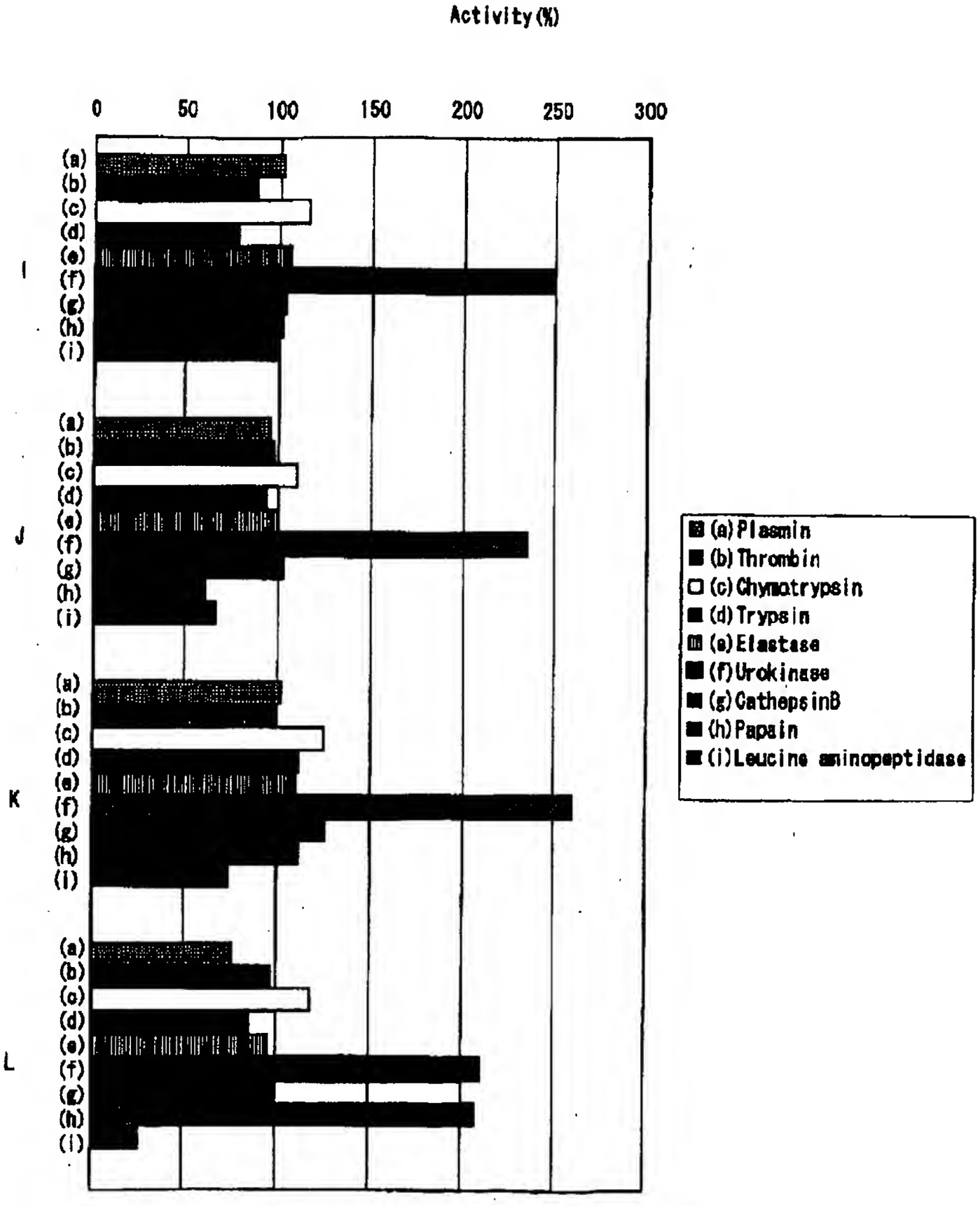

図3.　合成ペプチドの各種プロテアーゼ活性化作用

【図１】

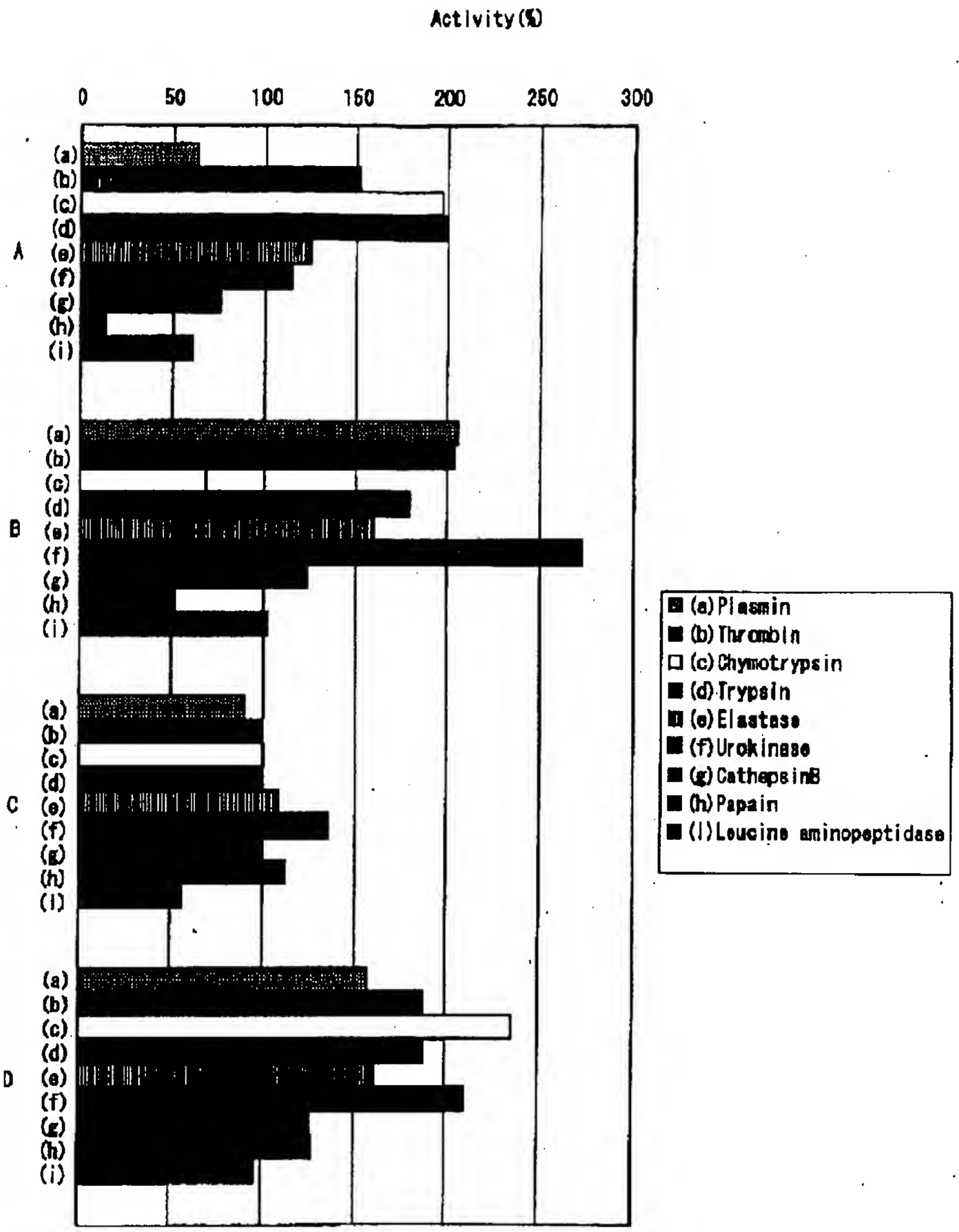

図１．　合成ペプチドの各種プロテアーゼ活性化作用

【図２】

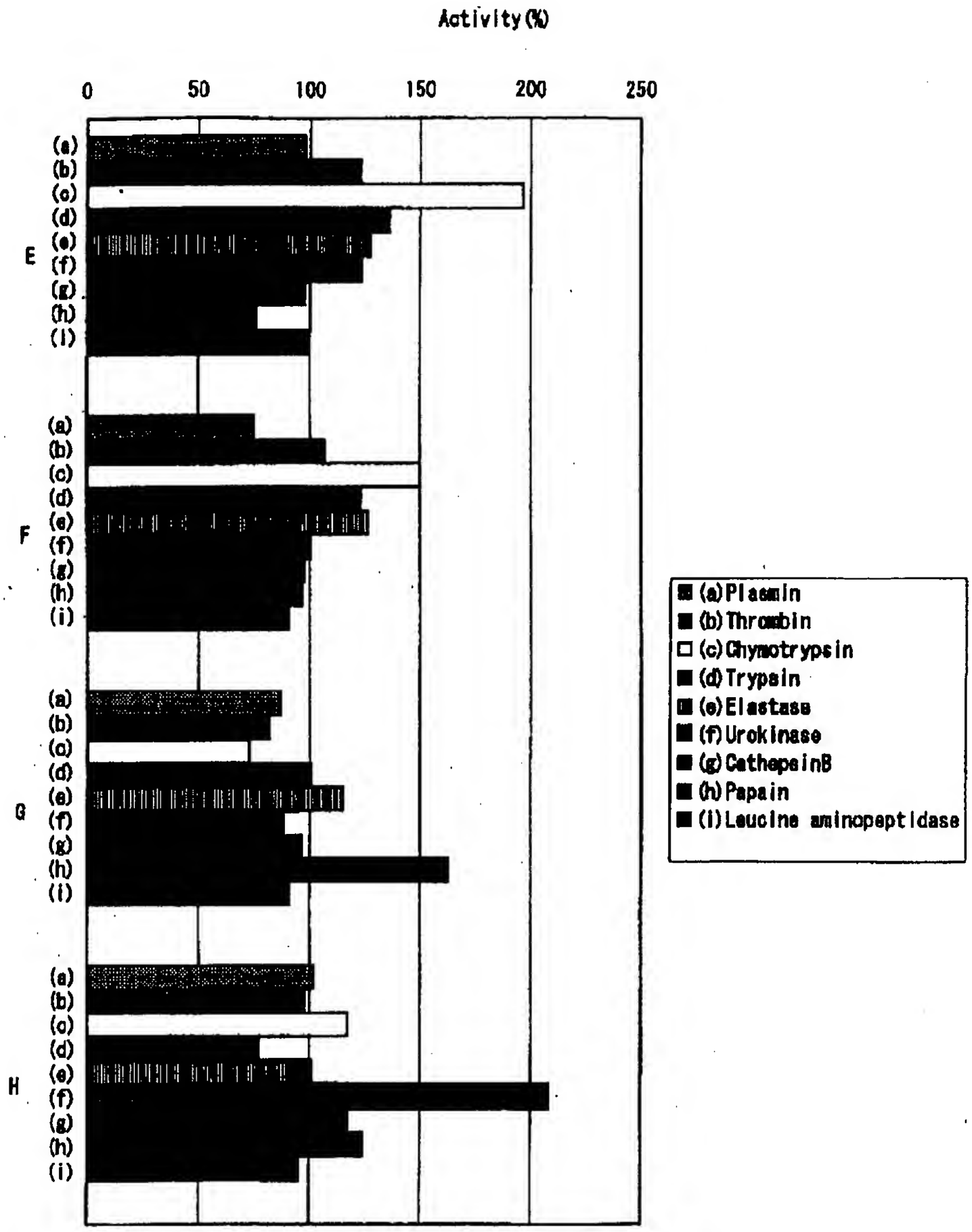

図 2.　合成ペプチドの各種プロテアーゼ活性化作用

【図４】

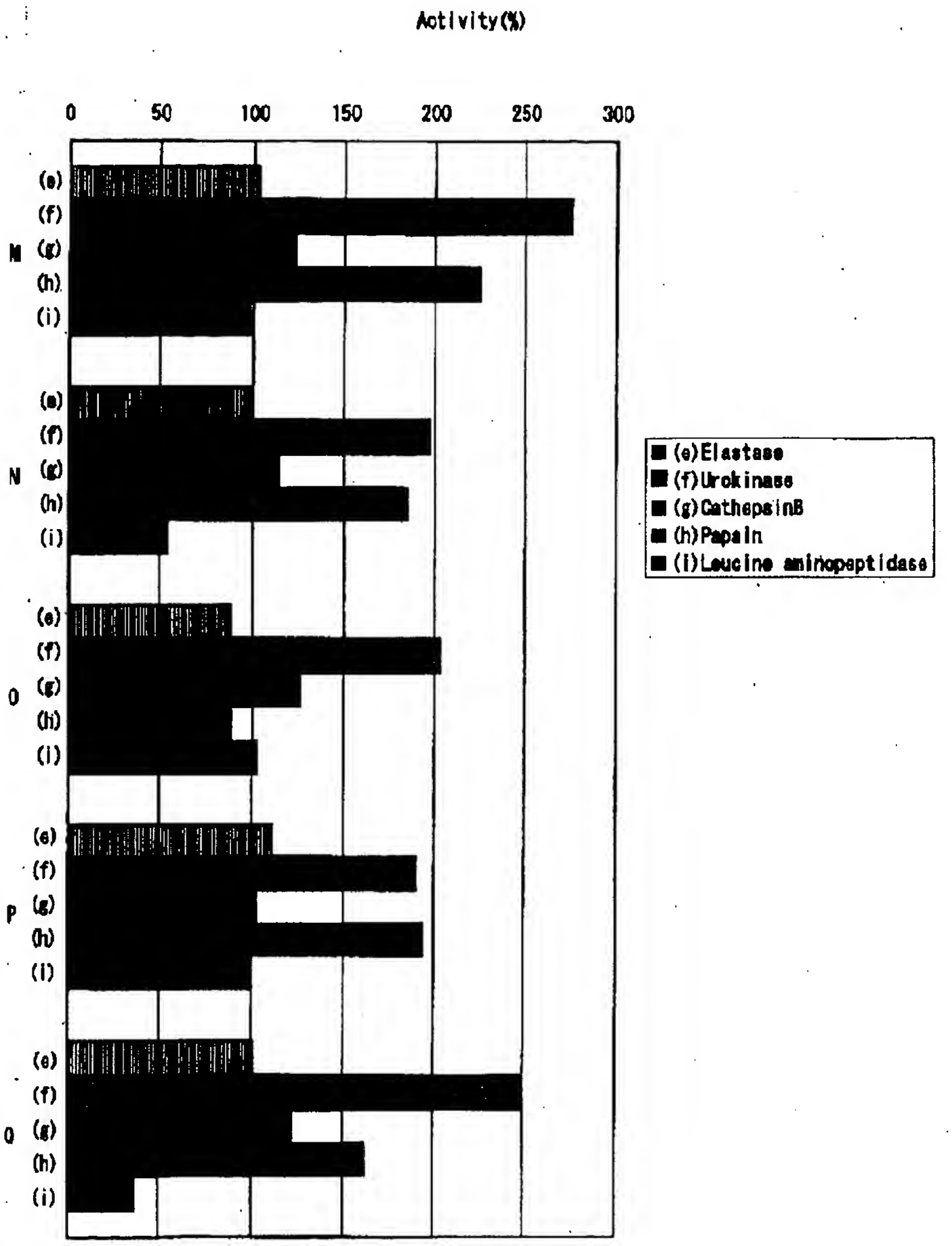

図４．　合成ペプチドの各種プロテアーゼ活性化作用

フロントページの続き

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* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] Tripeptide shown by Pro-A-B (however, A=Phe, Lys, Asn and Tyr, Thr;B=Pro, and Trp are shown.), or protease activator which contains those salts accepted physiologically as an active principle.
[Claim 2] Tripeptide shown by C-D-Pro (however, C=Tyr, Glu, Pro;D=Asn, and Ser, Arg and Tyr are shown), or protease activator which contains those salts accepted physiologically as an active principle.
[Claim 3] Tripeptide shown by Thioproline-Thr-Trp, or protease activator which contains those salts accepted physiologically as an active principle.
[Claim 4] Protease activator which contains the dipeptides shown by Glu-Arg, or those salts that were accepted physiologically as an active principle.
[Claim 5] Protease activator given in any 1 term of the 1st term of a patent claim characterized by being the derivatives by which the amino terminal of tripeptide was acylated, or those salts that were accepted physiologically - 4 term.
[Claim 6] Protease activator given in any 1 term of the 1st term of a patent claim characterized by being the derivatives with which the C terminal of tripeptide was amidated, or those salts that were accepted physiologically - 4 term.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the constituent containing the peptide which has activity useful as protease activator.
[0002]
[Description of the Prior Art] Research of the inhibitor of thrombin activity is known about using the tripeptide and its derivative as drugs (journal medical chemistry: vol.37, p.2122, 1994 year **:vol.36, p. 1993 [300 or]). Moreover, the development research as drugs of a comparatively short peptide and its derivative is seen by for example, a ****** No. 502154 [four to] official report, a ****** No. 502306 [four to] official report, a ****** No. 502308 [four to] official report, a ****** No. 502309 [four to] official report, etc. In these researches, a prolyl radical is contained in the amino acid sequence of the peptide, and the number of amino acid of the die length of an array is five or more. These peptides can also be said to be the TNF amelioration peptide guided from the tumor necrosis factor (TNF). Thus, some researches which use a short peptide as drugs were made. that intracellular is easy to be incorporated considers a short peptide enough -- having -- in addition -- and since it is decomposed in the living body and they serve as harmless amino acid, it is thought that the side effect over administration to a living body is hardly produced. Therefore, it is thought that these short peptides begin cancer and can serve as promising drugs as a remedy of other diseases in the future.
[0003] Conventionally, about activation of a protease, many reports have been made as one of the reactions in the field of inflammation. However, many of these reports are generation of the plasmin by activation of the plasminogen according [the good example] to urokinase or a plasminogen activator about the precursor of a protease acting as an activity protease in response to activation. Although activation by the trypsin of a chymotrypsin etc. is one of those there is no example not much and were generally known well, if the retrieval research report of the matter which activates the protease itself on the other hand moreover serves as protease activator of a small peptide with low-molecular, there is an old place. [no]
[0004]
[Problem(s) to be Solved by the Invention] The object of this invention is to offer the short peptide which has the activity as protease activator, and the object of this invention still more specifically has it in offering the protease activator containing the tripeptide which has a specific amino acid sequence, dipeptides, or those derivatives, and it is for other objects of this invention to offer the remedy constituent containing the salt accepted physiologically [the tripeptide which has a specific amino acid sequence, a dipeptide, or its derivative].
[0005]
[Means for Solving the Problem] It traced that it was a protein-protein interaction that this invention persons have determined the important metabolic fate wholeheartedly among the metabolic fates which constitute the growth mechanism of a cancer cell as a result of examination, and since the peptide equivalent to the joint domain of the protein concerning this interaction was able to control the function

of this protein, I thought that such a short peptide was useful as an anticancer agent.
[0006] Then, various oncogene products in order that the amino acid sequence of promising tripeptide may predict, For example K-Sam, Yes, Ret, Kit and Fms, ErbB, Met, Ros, Sea, Trk, Src, Fgr, Fyn, Lyn, Lck, Hck, Abl, and Arg etc. -- the consensus sequence of the oncogene product which has the array further called the Sark homology (SH) domain was searched, and it searched for the tripeptide thru/or the dipeptide which predicts those junction sequences and is equivalent to the domain. Consequently, as tripeptide, Pro-Phe-Pro, Pro-Lys-Pro, Pro-Asn-Pro, Pro-Tyr-Pro, Tyr-Asp-Pro, Tyr-Ser-Pro, Glu-Arg-Pro, Pro-Tyr-Trp, Thiopro(Thioproline)-Thr-Trp and such N-acetyl object, or C-amide object was found out, and Glu-Arg, D-Glu-Arg, and Glu-D-Arg were found out as a dipeptide. Although their eyes were turned to the drug retrieval by preventing proteinic association in the above research, it found out that some which have the activity as protease activator were in the peptide compounded to the completely unexpected thing. This invention is made based on the above knowledge.
[0007] That is, this invention is 1. Tripeptide shown by Pro-A-B (however, A=Phe, Lys, Asn and Tyr, Thr;B=Pro, and Trp are shown.), Or the protease activator which contains those salts accepted physiologically as an active principle, 2. Tripeptide shown by C-D-Pro (however, C=Tyr, Glu, Pro;D=Asn, and Ser, Arg and Tyr are shown), Or the protease activator which contains those salts accepted physiologically as an active principle, 3. Tripeptide shown by Thioproline-Thr-Trp, Or protease activator, 4. which contain those salts accepted physiologically as an active principle The protease activator which contains the dipeptides shown by Glu-Arg or those salts that were accepted physiologically as an active principle is offered.
[0008]
[Embodiment of the Invention] As an amino acid sequence of the tripeptide concerning this invention, they are Pro-Phe-Pro, Pro-Lys-Pro, Pro-Tyr-Pro, Tyr-Ser-Pro, Glu-Arg-Pro, Pro-Tyr-Trp, etc. As an amino acid sequence of the dipeptide concerning this invention, it is Glu-Arg etc.
[0009] In this invention, that by which the amino acid of the amino terminal of the above-mentioned tripeptide and a dipeptide was acylated, the thing by which the amino acid of a C terminal was amidated, and the thing with which ends were embellished like the above are also used.
[0010] As an N-acylation derivative of the tripeptide of this invention, and a dipeptide, it is a formyl group, an acetyl group, an aryl carbonyl group, and an aromatic series carbonyl group derivative, and the amino group, an alkylamino radical, and an aromatic series amino-group derivative can be raised as a C-amidation derivative.
[0011] As a salt accepted physiologically [the tripeptide of this invention, a dipeptide, and its derivative], a hydrochloric acid, a citric acid, a phosphoric acid, a tartaric acid, a lactic acid, an acetic acid, a formic acid, a fumaric acid, a maleic acid, a succinic acid, etc. are raised.
[0012] Composition of the tripeptide concerning this invention, a dipeptide, and its derivative is the Japanese Biochemical Society edit ******** experiment lecture 2. As proteinic chemistry (below) has a publication, it is easily compoundable by any approach of a solid phase technique or a liquid phase process.
[0013] Purification of the tripeptide concerning this invention, a dipeptide, and its derivative can be performed by the usual approaches, such as a silica gel column chromatography and an ion-exchange column chromatography. There is no definition also in an eluate and it can use the usual organic solvents, such as water, a methanol, and chloroform, for it.
[0014] It is 1-ethyl about the amino acid of the commercial item which protected the carboxyl group by benzyl (Bzl radical) first when describing composition of the tripeptide concerning this invention, a dipeptide, and its derivative in more detail. - 3 -(3-dimethylaminopropyl)- It condenses using condensing agents, such as a carbodiimide hydrochloride (WSCD HCl) and dicyclohexylcarbodiimide (DCCD), and a dipeptide is obtained. As a solvent used for the condensation reaction of the tripeptide of this invention, and a dipeptide, N.N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), an acetonitrile, 1,4-dioxane, tetrahydro furans (THF), and such mixture are suitable. Clearance of the protective group of tripeptide and a dipeptide can use the hydrogen catalytic reduction method or liquefaction hydrogen fluoride (HF) which made the catalyst a trifluoro methansulfonic acid method

(TMSFA law), and palladium/carbon. Generally as a solvent used with a hydrogen catalytic reduction method, it is used, and is satisfactory. A methanol (it may outline Following MeOH), acetic acids, or such mixture are suitable. The general approach of thin-layer chromatography (TLC) can be used for the reaction progress in each synthetic step, or the check of purity. In an expansion solvent, the check of a spot can use the hydrogen bromide (HBr)-ninhydrin method using a chloroform-methanol system and an n-butanol-acetic-acid-pyridine-water (4:1:1:2) system.

[0015] Although there is especially no constraint except the protease activator of this invention containing the tripeptide obtained as mentioned above, a dipeptide and its derivative, or those salts that were accepted physiologically as an active principle, the additive usually used on the occasion of pharmaceutical-preparation-izing of a compound may also be included.

[0016] Although the origin and a class are not asked, it is the thing of the Homo sapiens origin preferably, and plasmin, a thrombin, a chymotrypsin, a trypsin, elastase, urokinase, cathepsin B, a papain, and a leucine aminopeptidase are [that what is necessary is just a protease as a protease which the protease activator of this invention can activate] desirable as a protease.

[0017] This invention is not limited by this although an example explains this invention concretely below.

[0018]

[Example]

Example 1 [Composition of Pro-Phe-Pro (PFP)]

Boc-Phe 2.7g (10 millimol), ProOBzl HCl 2.4g (10 millimol), and 1-hydroxy benzotriazol (HOBt) 1.5g (11 millimol) were dissolved in DMF 30ml, and WSCD HCl 1.7g (11 millimol) suspended in DMF 10ml was dropped at the bottom of ice-cooling stirring. triethylamine (TEA) -- after adding 1.8ml and checking that it is pH 7-8 with the omnipotent pH indicator paper, it stirred at the room temperature all night. After filtering and removing the precipitation which deposited and carrying out vacuum concentration of the filtrate, 300 ml ethyl acetate (EtOAc) was added and it washed in order of 10% salt (NaCl) water solution of 4% sodium hydrogencarbonate (NaHCO3), 10%NaCl water solution, 10% NaCl water solution of 0.4M citric acid, and 10%NaCl water solution. Anhydrous sodium sulfate (Na2SO4) was added to the EtOAc layer, reduced pressure distilling off of the solvent was carried out after dehydration, and reduced pressure hardening by drying of the residue was carried out in the desiccator. It is 4 Ns to this hardening-by-drying object. A hydrochloric acid/dioxane 40ml, thioanisole 4ml was added and it reacted under ice-cooling for 2 hours. Dioxane after carrying out vacuum concentration of this The actuation which adds and condenses 50ml was repeated twice, and the actuation which adds and condenses diethylether (Et2O) similarly was repeated twice. Z-Pro 1.5g (6.2 millimol) and HOBt 0.84g (6.8 millimol) were added to the hardening-by-drying object which carried out reduced pressure hardening by drying of this, and it dissolved in it at DMF 30ml. WSCD HCl 1.2g (6.8 millimol) suspended in DMF 10ml was dropped under ice-cooling stirring. After preparing to pH 7-8 by TEA 1.6ml, it was made to react at a room temperature for 1.5 hours. After making it react at 4 more degrees C all night, the precipitation which deposited was filtered and removed and vacuum concentration of the filtrate was carried out. EtOAc 300ml was added and it washed in order of 10% NaCl water solution of 4%NaHCO3, 10%NaCl water solution, 10%NaCl water solution of 0.4M citric acid, and 10%NaCl water solution. Anhydrous Na2SO4 was added to the EtOAc layer, reduced pressure distilling off of the solvent was carried out after dehydration, and reduced pressure hardening by drying of the residue was carried out in the desiccator. A hardening-by-drying object is dissolved in a small amount of methanol (MeOH), and it is sephadex. Separation purification was carried out in LH-20 column (bore [of 3cm] x die length of 55cm). It was eluted with this solvent and fractionation was carried out in 5 minutes (about 8ml) per one fraction. By TLC (Kieselgel 60F254 the system of chloroform-MeOH was used for the expansion solvent.), after the check, the fractions containing the specified substance were collected and reduced pressure hardening by drying was carried out about each fraction. This was dissolved in the mixed liquor of MeOH 20ml and a 20 ml acetic acid (AcOH), and it stirred under the nitrogen air current. It is stirring A stop and 10% After adding palladium / carbon (Pd/C) (51.60% hydrated compound) 2.0g and leading a hydrogen air current, it stirred violently by

ordinary temperature ordinary pressure, and the reaction was started. After it permuted the stop in the place which absorption of hydrogen ended and nitrogen gas permuted hydrogen gas for stirring, the catalyst was carried out the ** exception. Water was added to what carried out vacuum concentration of the filtrate, and vacuum concentration was carried out once again. It is sephadex about a concentrate. Separation purification was carried out in G-10 column (bore [of 2.6cm] x die length of 60cm). It was eluted with water and fractionation was carried out in 5 minutes (about 10ml) per one fraction. The purity of each fraction was checked by TLC (n-butanol:acetic-acid:pyridine:water =4:1:1:2 (BAPW system) was used for the expansion solvent.) (Rf value 0.39), the fractions which give the spot of the specified substance individually were collected, and reduced pressure hardening by drying was carried out. This hardening-by-drying object is dissolved in 15ml water, and it freeze-dries, and is the colorless amorphous-like specified substance. 1.0g It obtained. The overall yield so far was 28%. Elemental analysis C19H25N3O4 MW.359.43 Calculated value C 63.49, H 7.01, N 11.69. Analysis value C 59.41, H 7.42, N 10.66.

[0019] Example 2 [Composition of N-acetylation object (Ac-PFP) of Pro-Phe-Pro]

Pro-Phe-Pro 1.2g (3.3 millimol) obtained in the example 1 is dissolved in 10ml of water, and it is this. N-acetyl succinimide (N-ASI) 1.5g (10 millimol) is added, and it is 1 N. pH was stirred at the room temperature after preparation to about 7 with the potassium-hydroxide water solution all night. Sephadex after condensing reaction mixture Separation purification was carried out in G-10 column (bore [of 2.6cm] x die length of 60cm). The colorless amorphous-like specified substance which will give a single spot (expansion solvent BAPW system Rf=0.56) by TLC if each fraction is checked by TLC and concentration hardening by drying is carried out 1.2g (90% of yield) was obtained. Elemental analysis C21H27N3O5 MW.401.46 Calculated value C 62.83, H 6.78, N 10.47. Analysis value C 59.69, H 6.50, N 10.97.

[0020] Example 3 [Composition of C-amidation object (PFP NH2) of Pro-Phe-Pro]

Boc-Phe 2.3g (8.8 millimol), Pro NH2 1.0g (8.8 millimol), and HOBt 1.33g (9.8 millimol) were dissolved in DMF 30ml, and it suspended in DMF 10ml under ice-cooling stirring. WSCD HCl (MW115.24 36.46) 1.7g (8.8 millimol) was dropped. After checking that it is pH 7-8 with the omnipotent pH indicator paper [TEA 1.7ml], it was made to react at 4 degrees C all night. It processed like the example 1 after the reaction, and Boc-Phe-Pro NH2 was obtained as colorless oily matter. It is 4 Ns to this. A hydrochloric acid/dioxane 16ml, thioanisole 1.5ml was added and it stirred under ice-cooling for 1 hour. It processed like the example 1, Z-Pro 2.0g (7.9 millimol) and HOBt 1.1g (8.8 millimol) were added to the obtained residue, and it dissolved in DMF 20ml. WSCD HCl 1.7g (8.7 millimol) suspended in DMF10ml was dropped under ice-cooling stirring. After preparing to pH 7-8 by TEA 1.5ml, it was made to react at 4 degrees C all night. The same processing as an example 1 was performed after the reaction, and the oily matter of Z-Pro-Phe-Pro NH2 was obtained. It is sephadex like an example 1. LH-20 column refines, and by TLC, a single spot is obtained mostly, it continues, and they are after a deprotection reaction and sephadex by the hydrogen catalytic reduction method. G-10 column refines, and it is made to crystallize from Et2O, and is a colorless crystal. 0.2g (BAPW system Rf=0.48) was obtained. The overall yield so far was 39%. Elemental analysis C19H26N4O3 MW.358.44 Calculated value C 60.37, H 7.31, N 15.63. Analysis value C 60.48, H 7.38, N 14.55.

[0021] Example 4 [Composition of N-acetylation of Pro-Phe-Pro, and C-amidation object (Ac-PFP NH2)]

It is the specified substance by adding 6ml of water, dissolving in PFP NH2 1.0g (2.8 millimol) obtained in the example 3, and reacting and refining like an example 2. 0.9g (80% of yield) was obtained. This gave the single spot (expansion solvent BAPW system Rf=0.62) by TLC. Elemental-analysis C21H28N4O4 MW.400.25 Calculated value C 62.98, H 7.05, N 13.99. Analysis value C58.91, H6.68, N13.01.

[0022] Example 5 [Composition of Pro-Lys-Pro (PKP)]

It dissolved in 400ml EtOAc and Boc-Lys (Cl-Z) t-Bu NH2 3.5g (7.1 millimol) was washed in order of 10%NaCl water solution of 0.4M citric acid, and 10%NaCl water solution. Anhydrous Na2SO4 is added to an EtOAc layer, reduced pressure distilling off of the solvent is carried out after dehydration, and it is

the shape of amorphous. Boc-Lys (Cl-Z) was obtained. ProOBzl HCl 1.7g (7.1 millimol), and HOBt 1.0g (7.7 millimol) and WSCDHCl 1.5g (7.8 millimol) were added to this, it reacted and refined like the example 1, and Boc-Lys(Cl-Z)-ProOBzl was obtained. Acid treatment of the obtained hardening-by-drying object was carried out like the example 1, the Boc radical was removed, Z-Pro 1.7g (6.8 millimol), HOBt 1.0g (7.5 millimol), and WSCD HCl 1.5g (7.8 millimol) were added to the obtained residue, it reacted and refined like the example 1, and oil-like Z-Pro-Lyz(Cl-Bzl)-ProOBzl was obtained. Furthermore, it is sephadex about this. LH-20 column (bore [of 2.6cm] x die length of 60cm) refined, the fractions which give an almost single spot by TLC were collected, and reduced pressure hardening by drying was carried out. Et2O melted to little EtOAc was added to this, and it crystallized by cooling. A crystal is separated, reduced pressure hardening by drying is carried out in a vacuum desiccator, and it is a colorless crystal. 2.68g was obtained. This crystal was dissolved in AcOH 36ml and MeOH 24ml, and all protective groups were removed with the hydrogen catalytic reduction method like the example 1. Sephadex Separation purification was carried out in G-10 column (bore [of 2.6cm] x die length of 60cm). When each fraction is checked by TLC (expansion solvent BAPW system Rf=0.009), the target fraction is collected and condensed and it freeze-dries further, it is the colorless amorphous-like specified substance. 1.4g was obtained. The overall yield so far was 63%. Elemental analysis C16H28N4O4 MW.340.42 Calculated value C 56.45, H 8.29, N 16.34. Analysis value C54.06, H8.59, N15.27.

[0023] Example 6 [Composition of N-acetylation object (Ac-PKP) of Pro-Lys-Pro]
Amorphous-like object of Pro-Lys-Pro obtained in the example 5 1.2g (3.8 millimol) was dissolved in 10ml of water, N-ASI 1.6g (11.3 millimol) was added after adjusting a solution to pH7, and it stirred at the room temperature overnight. After carrying out concentration hardening by drying of the reaction mixture, residue is dissolved in a small amount of chloroform (CHCl3). CHCl3: Silica gel produced with the solution of MeOH=10:1 In C-200 column (bore [of 2.2cm] x die length of 33cm), by 300ml of solutions of CHCl3:MeOH=10:1 after regular placing And you made it eluted only in MeOH and fractionation was carried out to about 10ml fraction, each fraction was checked by TLC, even the fractions 102-115 which give the spot of the specified substance were collected, and concentration hardening by drying was carried out. Colorless amorphous-like object which will give a single spot (expansion solvent BAPW system Rf=0.40) by TLC after dissolving this in little water if it freeze-dries 1.25g (76%) It was obtained. It was satisfied with the nuclear-magnetic-resonance spectrum (NMR) of the location of a signal. 1H NMR(DMSO-d6, delta) 1.82 (3H, s) 2.15 (3H, s).

[0024] Example 7 [Composition of Pro-Lys-Pro NH2 (PKP NH2)]
Boc-Lys (Z) 3.5 g (9.2 millimol), Pro NH2 1.1g (9.2 millimol), HOBt 1.34g (9.9 millimol), and WSCD HCl 1.95g (10 millimol) are reacted and refined like an example 1, and it is the specified substance. Boc-Lys(Z)-Pro NH2 was obtained as colorless oily matter. To the residue which processed like the example 1 and was obtained, Z-Pro 1.5g (6.1 millimol), HOBt 0.85g (6.3 millimol) and WSCD HCl 1.3g (6.8 millimol) are added. It reacts like the approach indicated in the example 1, Z-Pro-Lys-Pro NH2 is obtained as oily matter, and it is sephadex like an example 1 about this. What refines in LH-20 column and gives an almost single spot by TLC was obtained. Then, it dissolves in little water after a deprotection reaction with the hydrogen catalytic reduction method using Pd/C 10%, and is sephadex. The specified substance which will give a single spot (expansion solvent BAPW system Rf=0.17) if it refines and freeze-dries in G-10 column 1.1g was obtained. The overall yield so far was 35%. C16H29N5O3 MW 339.44 Calculated value C 56.62, H 8.61, N 20.63. Analysis value C 51.56, H 8.83, N 18.63.

[0025] Example 8 [Composition of Pro-Tyr-Pro (PYP)]
Boc-Tyr (Bzl) 2.0g (5.3 millimol), ProOBzl HCl 1.3g (5.38 millimol), HOBt 0.81g (6.0 millimol), and WSCD HCl 1.14g (5.9 millimol) are made to react like an example 1, and it is the specified substance. Boc-Tyr(Bzl)-ProOBzl was obtained. To the residue which carried out acid treatment to this hardening-by-drying object like the example 1, removed the Boc radical, and was obtained Sephadex after dropping Z-Pro 1.3g (5.1 millimol), HOBt 0.76g (5.6 millimol), and WSCD HCl 1.1g (5.6 millimol) and making it react like an example 1 Separation purification was carried out in LH-20 column (bore [of

2.6cm] x die length of 60cm). It is little EtOAc about this after carrying out vacuum concentration. It dissolved, Et2O was added and it crystallized by cooling. The crystal was separated, reduced pressure hardening by drying was carried out, and 2.68g was obtained. Sephadex after carrying out deprotection of this crystal with a hydrogen catalytic reduction method G-10 The column refined. Dissolved in little MeOH after concentration, add Et2O and it was made to crystallize, the crystal after cooling was carried out the ** collection, and 1.16g was obtained when it dried. The overall yield so far was 55%. This thing gave the single spot (expansion solvent BAPW system Rf=0.32) by TLC. Elemental analysis C19H25N3O5 MW375.43 Calculated value C 60.79, H 6.71, N 11.19. Analysis value C 57.54, H 6.94, N 10.19.

[0026] Example 9 [Composition of N-acetylation object (Ac-PYP) of Pro-Tyr-Pro]

Add N-ASI 1.1g (8 millimol) to Pro-Tyr-Pro 1g (2.6 millimol) obtained in the example 8, and it is made to react like an example 2, and is chloroform about reaction mixture. The actuation extracted by 20ml was repeated 5 times. After [dehydration] reduced pressure hardening by drying of the chloroform layer was carried out by Na2SO4. Colorless amorphous-like object which dissolves this in little water, freeze-dries, and gives a single spot (expansion solvent BAPW system Rf=0.51) by TLC 0.68g (61% of yield) was obtained. Elemental analysis C21H27N3O6 MW417.46 Calculated value C 60.42, H 6.52, N 10.07. Analysis value C 54.52, H 6.58, N11.04.

[0027] Example 10 [Composition of Pro-Tyr-Pro NH2 (PYP NH2)]

WSCD HCl 1.7g (9.0 millimol) was added to Boc-Tyr (Bzl) 3.25g (8.8 millimol), Pro NH2 1.0g (8.8 millimol), and HOBt 1.2g (8.9 millimol), it reacted and refined like the example 1, and Boc-Tyr(Bzl)-Pro NH2 was obtained as colorless oily matter. Acid treatment of the obtained hardening-by-drying object was carried out like the example 1, the Boc radical was removed, vacuum concentration of the solvent was carried out, Et2O was added, and the crystal was deposited. This crystal was separated and reduced pressure hardening by drying was carried out in the vacuum desiccator. Z-Pro 2.0g (7.9 millimol) and HOBt 1.2g (8.9 millimol) were added to the crystal which hardened by drying, WSCD HCl 1.7g (9.0 millimol) was dropped, it reacted and refined like the example 1, and oily matter was obtained. This was dissolved in a small amount of methanol, and it applied to sephadex LH-20 column (bore [of 2.6cm] x die length of 60cm), and was eluted with this solvent. After looking for the target fraction in TLC and bringing together in one, reduced pressure hardening by drying was carried out, and 4.9g of amorphous-like specified substance was obtained. Sephadex after performing the deprotection reaction according this to a hydrogen catalytic reduction method The colorless amorphous-like specified substance which freeze-dries after purification in G-10 column, and gives a single spot (expansion solvent BAPW system Rf=0.42) by TLC 2.9g was obtained. The overall yield so far was 87%. Elemental analysis C19H26N4O4 MW 377.44 calculated value C 60.95, H 7.60, N14.96. Analysis value C 56.07, H 7.19, N 13.84.

[0028] Example 11 [Composition of N-acetylation of Pro-Tyr-Pro, and C-amidation object (Ac-PYP NH2)]

When add N-ASI 1.0g (7 millimol) to PYP NH2 1.0g (2.6 millimol) obtained in the example 10, react and refine like an example 2, the fractions which give a single spot (expansion solvent BAPW system Rf=0.61) mostly by TLC are collected, it condenses and it freeze-dries, it is the colorless amorphous-like specified substance. 1.05g was obtained. The yield so far was 96%. Elemental analysis C21H28N4O5 MW 416.48 Calculated value C 60.56, H 6.78, N 13.45. Analysis value C 55.11, H 6.01, N 13.09[0029] Example 12 [Composition of Tyr-Ser-Pro (YSP)]

Having dissolved in DMF 20ml and stirring Boc-Ser (Bzl) 2.0g (6.8 millimol), ProOBzl HCl 1.6g (6.8 millimol), and HOBt 1.0g (7.5 millimol) under ice-cooling, it was made to react like the approach indicated in the example 1 in addition to WSCD HCl 1.4g (7.3 millimol) suspended in DMF 10ml, and Boc-Ser(Bzl)-ProOBzl was obtained. Perform the deBoc radical reaction by acid treatment like the approach indicated in the example 1, add Z-Tyr (Bzl) 2.6g (6.3 millimol) and HOBt 0.85g (6.3 millimol) to the obtained residue, WSCD HCl 1.3g (6.8 millimol) is dropped, it is made to react like an example 1, the obtained residue is melted to little MeOH, and it is sephadex. LH-20 column refined. The target fraction was looked for in TLC, and were collected, and reduced pressure hardening by drying was

carried out. After dissolving this in EtOAc 50ml, 250ml Et2O was added and it crystallized by leaving it in a refrigerator. The crystal was separated and reduced pressure hardening by drying was carried out. (2.40g) . The deprotection reaction according this crystal to a hydrogen catalytic reduction method was performed. Sephadex The colorless amorphous-like specified substance which will give a single spot (expansion solvent BAPW system Rf=0.30) by TLC if it refines and freeze-dries in G-10 column 1.70g was obtained. The overall yield so far was 68%. Elemental analysis C17H23N3O6 MW 365.38 Calculated value C 56.03, H 6.63, N15.37. Analysis value C 55.91, H 6.81, N 13.82.

[0030] Example 13 [Composition of N-acetylation object (Ac-YSP) of Tyr-Ser-Pro]

N-ASI 1.1g (8 millimol) was added to YSP 1.0g (YSP 2.7 millimol) obtained in the example 12, and it was made to react at a room temperature for 2 hours (pH 8 [about]). Sephadex after condensing reaction mixture The specified substance which refines in G-10 column and gives a single spot (expansion solvent BAPW system Rf=0.44) by TLC 0.6g was obtained. The overall yield so far was 54%. Elemental analysis C19H25N3O7 MW 407.41 Calculated value C 56.01, H 6.18, N 10.31. Analysis value C 54.95, H 5.37, N 10.11.

[0031] Example 14 [Composition of Tyr-Ser-Pro NH2 (YSP NH2)]

Added WSCD HCl 2.0g (10 millimol) to Boc-Ser (Bzl) 2.6g (8.8 millimol), Pro NH2 1.0g (8.8 millimol), and HOBt 1.30g (9.7 millimol), it was made to react like an example 1, and Boc-Ser(Bzl)-Pro NH2 was obtained as oily matter. After carrying out acid treatment like the approach which indicated this oily matter in the example 1 and condensing a solvent, Et2O was added and precipitation was obtained in the decantation. Reduced pressure hardening by drying of this was carried out, Z-Tyr(Bzl) 3.2g (7.9 millimol) and HOBt 1.2g (8.7 millimol) were added, and WSCDHCl 1.7g (8.8 millimol) suspended in DMF was dropped. It dissolved in little EtOAc after condensing a solvent, Et2O was added, and it crystallized by cooling. 3.25g was obtained when reduced pressure drying of the crystal was separated and carried out. A deprotection reaction is performed for this crystal with a hydrogen catalytic reduction method, and it is sephadex. When the fraction of the specified substance which refines in G-10 column and gives an almost single spot (expansion solvent BAPW system Rf=0.44) by TLC is collected and condensed and it freeze-dries, it is a colorless amorphous-like object. 1.7g was obtained. The overall yield so far was 53%. Elemental analysis C17H24N4O5 MW 364.39 Calculated value C 56.03, H 6.63, N 15.37. Analysis value C 53.91, H 6.71, N13.82.

[0032] Example 15 [Composition of N-acetylation of Tyr-Ser-Pro, and C-amidation object (Ac-YSP NH2)]

N-ASI 1.0g (7 millimol) was added to YSP NH2 0.8g (2.2 millimol) obtained in the example 14, and it was made to react like the approach indicated in the example 2, and processed, and the hardening-by-drying object was obtained. However, since mixing of a condensing agent origin object was presumed by this, it dissolved in little water, and when the extract was repeated 5 times by EtOAc 10ml and the water layer was freeze-dried, 0.87g (yield 95%) of colorless amorphous-like objects was obtained by it. This thing gave the single spot (expansion solvent BAPW system Rf=0.56) by TLC.

[0033] Example 16 [Composition of Glu-Arg-Pro (ERP)]

WSCD HCl 2.1g (11.0 millimol) was added to Boc-Arg (NO2) 1/2AcOEt 1/4H2O 3.7g (10 millimol), ProOBzl HCl 2.4g (10 millimol), and HOBt 1.5g (11 millimol), and it was made to react like an example 1, and processed, and Boc-Arg(NO2)-ProOBzl was obtained as oily matter. Acid treatment of the obtained oily matter was carried out like the example 1. Z-Glu(Bzl) 1.7g (5.0 millimol), HOBt 0.75g (5.7 millimol), and WSCD HCl 1.1g (5.5 millimol) were dropped at the obtained oily matter, and it reacted to it like the example 1. After dissolving the obtained residue in little EtOAc, it crystallized, when Et2O was added and it cooled. The crystal was separated, reduced pressure hardening by drying was carried out, and the 2.3g crystal was obtained. It is sephadex about the residue which obtained this crystal by carrying out in the clearance reaction of all guard chambers with the hydrogen catalytic reduction method. G-10 column refines, and the fraction which gives a single spot (expansion solvent BAPW system Rf=0.007) by TLC is collected and condensed, and it freeze-dries, and is the colorless amorphous-like specified substance. 1.3g was obtained. The overall yield so far was 32%. Elemental analysis C16H28N6O6 MW 400.44 Calculated value C 47.99, H 7.05, N 20.99. Analysis value C 45.84,

H 7.55, N 19.52.
[0034] Example 17 [Composition of N-acetylation object (Ac-ERP) of Glu-Arg-Pro]
It was obtained in the example 16. N-ASI 1.1g (7.1 millimol) was added to ERP 1.1g (2.7 millimol), and it stirred at the room temperature overnight. Sephadex after condensing reaction mixture G-10 A column refines, and each fraction is collected and condensed, and it freeze-dries, and is the colorless amorphous-like specified substance. 0.80g was obtained. The overall yield so far was 67%. This thing gave the single spot (expansion solvent BAPW system Rf=0.16) by TLC. Elemental analysis C18H30N6O7 MW442.47 Calculated value C48.86, H6.83, N18.99. Analysis value C45.89, H7.03, N18.07.
[0035] Example 18 [Composition of C-amidation object (ERP NH2) of Glu-Arg-Pro]
WSCD HCl 2.1g (11 millimol) was added to Boc-Arg (NO2) 1/2AcOEt 1/4H2O 3.7g (10.0 millimol), ProOBzl HCl 2.4g (10 millimol), and HOBt 1.49g (11 millimol), it reacted like the example 1, and chloroform was used and processed instead of EtOAc. It became cloudy when EtOAc was added to the residue which carried out vacuum concentration of the chloroform layer. Furthermore Et2O was added, the decantation of the gum-like settlings obtained by leaving it overnight at 4 degrees C was carried out, they were obtained, and reduced pressure hardening by drying was carried out. Acid treatment of the obtained hardening-by-drying object was carried out like the example 1, the Boc radical was removed, the settlings produced during the reaction were obtained by the decantation, and reduced pressure hardening by drying was carried out. After having added Z-Glu (Bzl) 1.7g (5.0 millimol) and HOBt 0.75g (5.5 millimol) to this, dropping WSCD HCl 1.1g (5.7 millimol) and processing by reacting like an example 1, the column refined and colorless oily matter was obtained. Sephadex after performing the clearance reaction of all protective groups for this oily matter with a hydrogen catalytic reduction method G-10 column refined, the fractions of a single spot (expansion solvent BAPW system Rf=0.21) were mostly collected by TLC, and crystalline residue was obtained. Cold MeOH was added to this, and 0.2g was obtained when the ** collection of the crystal was unfolded and carried out. The overall yield so far was 6%. Elemental analysis C16H29N7O5 MW399.45 Calculated value C 48.11, H 7.32, N 24.55. Analysis value C 46.61, H 6.92, N22.34.
[0036] Example 19 [Composition of Pro-Thr-Trp (PTW)]
the DMF solution of Boc-ThrOSu 3.7g (12 millimol) and TrpOBzl HCl 3.8g (12 millimol) -- the bottom of ice-cooling stirring, and TEA -- in addition, it was made to react after checking that it is pH 7-8 overnight After treatment was carried out like the example 1, and Boc-Thr-TrpOBzl was obtained as oily matter. Then, acid treatment was carried out like the example 1, Z-Pro 2.8g (11 millimol) was added to the residue which might be removed in the Boc radical, the DMF 10ml solution of DCCD 2.5g (12 millimol) was dropped, and it was made to react at 4 degrees C overnight. Vacuum concentration of the filtrate is carried out the back according to **, settlings are processed like an example 1, and it is an amorphous-like object. 4.21g was obtained. Among these, the residue which removed 2g with the hydrogen catalytic reduction method, and was obtained in the protective group is dissolved in little water, and it is sephadex. The fractions which refine in G-10 column and give a single spot (expansion solvent BAPW system Rf=0.33) mostly by TLC were collected, and reduced pressure hardening by drying was carried out. Little MeOH is added to residue, it was crystallized, after cooling, the crystal was separated, it dried and 1.40g was obtained. The overall yield so far was 30%. Elemental analysis C20H25N4O5 MW 402.22 Calculated value C 59.69, H 6.51, N 13.91. Analysis value C 55.90, H 6.41, N 12.75.
[0037] Example 20 [Composition of Pro-Thr-Trp NH2 (PTW NH2)]
Boc-ThrOSu 6.0g (19 millimol) is dissolved in DMF 50ml, and it is Trp NH2 hydrochloride. 4.6g (19 millimol) and HOBt 2.9g (21 millimol) were added with powder, and the stirring dissolution was carried out. After adding TEA under ice-cooling stirring and checking that it is pH 7-8 WSCD HCl 4.1g (21 millimol) The DMF 10ml solution was added and it was made to react at 4 degrees C overnight. Precipitate is ****(ed) and it is filtrate after vacuum concentration. EtOAc 500ml was added and 8.3g (87%) was obtained by processing like an example 1 by making specified substance Boc-Thr-Trp NH2 into colorless oily matter. This is made to react like an example 1, and it processes, and is oily matter. 7.2g (16 millimol, 98%) was obtained. To then, this Z-Pro 4.1g (16 millimol), HOBt 2.5g (18 millimol),

and WSCD HCl 3.5g (18 millimol) were added, and it was made to react like an example 1, it processed, and 7.3g (70%) was obtained by using Z-Pro-Thr-Trp NH2 as an amorphous-like object. Pd/C 1g was added to 2.5g of this amorphous-like object 10%, and the deprotection reaction was performed after a reaction / processing like the example 1. What was obtained is dissolved in little water and it is sephadex. The specified substance which will consist of a single spot (expansion solvent BAPW system Rf=0.47) if it refines and freeze-dries in G-10 column 1.0g was obtained. Elemental analysis C20H27N5O4 MW.401.33 Calculated value C 59.80, H 6.78, N 17.46. Analysis value C 57.63, H 6.94, N 16.27.

[0038] Example 21 [Composition of N-acetylation object (Ac-PTW NH2) of Pro-Thr-Trp NH2]
After adding N-ASI 0.9g (6 millimol) to PTW NH2 0.95g (2.4 millimol) obtained in the example 20 and making it react at a room temperature overnight, concentration hardening by drying was carried out under reduced pressure. Silica gel which melted with a small amount of chloroform, and was produced by the mixture of chloroform:methanol =30:1 Regular placing is carried out to C-200 (Wako Pure Chem make) column (bore [of 2.6cm] x die length of 45cm), and it is chloroform:methanol =30:1 mixture. 400ml, 10:1 mixture Only 300ml and a methanol were made eluted in 500ml, and each fractions (about 10ml) were collected even for fractions 72-75 after the check by TLC. When residue is dissolved in little water and it freeze-dries after concentration hardening by drying, it is a colorless amorphous-like object. 0.8g was obtained. The overall yield so far is 75%, and is ****. This thing gave the single spot (expansion solvent BAPW system Rf=0.64) by TLC. Elemental analysis C22H29N5O3 MW443.50 Calculated value C 59.58, H 6.59, N 15.79. Analysis value C 56.98, H 6.54, N 15.34.

[0039] Example 22 [Composition of thioPro-Thr-Trp (P'TW)]
the DMF solution of Boc-ThrOSu 3.7g (12 millimol) and TrpOBzl HCl 3.8g (12 millimol) -- the bottom of ice-cooling stirring, and TEA -- in addition, it was made to react after checking that it is pH 7-8 overnight After treatment was carried out like the example 1, and Boc-Thr-TrpOBzl was obtained as oily matter. Then, acid treatment was carried out like the example 1, the Boc radical was removed, Boc-thioPro2.8g (12 millimol) was added to the obtained residue, the DMF 10ml solution of HOBt 1.784g (13.2 millimol) and WSCD HCl 2.53g (13.2 millimol) was dropped, and it was made to react at 4 degrees C overnight. Vacuum concentration of the filtrate is carried out the back according to **, settlings are processed like an example 1, and it is an amorphous-like object. 6.3g was obtained. Cutting of the protective group by HF of Boc-thioPro-Thr-TrpOBzl 6.3g obtained by coupling went as follows. Boc-thioPro-Thr-TrpOBzl 6.3g was equally divided into ten, and HF cut, respectively. That is, Boc-thioPro-Thr-TrpOBzl is taken in a reaction container, and it is thioanisole. 11ml was added and it put on the room temperature for 1 hour. It was made to react, stirring a reaction container by the dry ice-acetone bath and stirring HF 100ml at installation and 0 degree C under cooling for 1 hour. Reduced pressure clearance of the HF was carried out with the stream aspirator, and it was made to dry with a vacuum pump further. The obtained residue was dissolved by 100ml of acetic-acid water solutions 10%, and the thioanisole which washes twice and remains with 50ml diethylether was removed. The acetic-acid water-solution fraction was neutralized, 3.2g of things which carried out concentration hardening by drying was dissolved in little chloroform-methanol mixture (5:1), the silica gel column chromatography refined, and the specified substance was obtained. Yield of 0.6g. TLC (BAPW system Rf=0.62) gave the single spot. Elemental analysis C19H25N4O5S MW 420.53 Calculated value C 54.22, H 5.99, N 13.35. Analysis value C 55.90, H 6.41, N 12.75.

[0040] Example 23 [Composition of D-Pro-Thr-Trp NH2 (D-PTW NH2: Pro is D object)]

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TECHNICAL FIELD

[Field of the Invention] This invention relates to the constituent containing the peptide which has activity useful as protease activator.

[Translation done.]

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PRIOR ART

[Description of the Prior Art] Research of the inhibitor of thrombin activity is known about using the tripeptide and its derivative as drugs (journal medical chemistry: vol.37, p.2122, 1994 year **:vol.36, p. 1993 [300 or]). Moreover, the development research as drugs of a comparatively short peptide and its derivative is seen by for example, a ****** No. 502154 [four to] official report, a ****** No. 502306 [four to] official report, a ****** No. 502308 [four to] official report, a ****** No. 502309 [four to] official report, etc. In these researches, a prolyl radical is contained in the amino acid sequence of the peptide, and the number of amino acid of the die length of an array is five or more. These peptides can also be said to be the TNF amelioration peptide guided from the tumor necrosis factor (TNF). Thus, some researches which use a short peptide as drugs were made. that intracellular is easy to be incorporated considers a short peptide enough -- having -- in addition -- and since it is decomposed in the living body and they serve as harmless amino acid, it is thought that the side effect over administration to a living body is hardly produced. Therefore, it is thought that these short peptides begin cancer and can serve as promising drugs as a remedy of other diseases in the future.
[0003] Conventionally, about activation of a protease, many reports have been made as one of the reactions in the field of inflammation. However, many of these reports are generation of the plasmin by activation of the plasminogen according [the good example] to urokinase or a plasminogen activator about the precursor of a protease acting as an activity protease in response to activation. Although activation by the trypsin of a chymotrypsin etc. is one of those there is no example not much and were generally known well, if the retrieval research report of the matter which activates the protease itself on the other hand moreover serves as protease activator of a small peptide with low-molecular, there is an old place. [no]

[Translation done.]

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EFFECT OF THE INVENTION

[Effect of the Invention] By this invention, the completely new protease activator containing tripeptide, dipeptides, and those derivatives is offered. The active principle is a peptide, it is decomposed into amino acid and the protease activator of this invention is metabolized in the living body. Therefore, when a living body is medicated, there are very few dangers of causing a side effect. Therefore, it is possible to be effective as the drugs or the digestive accelerator for protease activity research.

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] The object of this invention is to offer the short peptide which has the activity as protease activator, and the object of this invention still more specifically has it in offering the protease activator containing the tripeptide which has a specific amino acid sequence, dipeptides, or those derivatives, and it is for other objects of this invention to offer the remedy constituent containing the salt accepted physiologically [the tripeptide which has a specific amino acid sequence, a dipeptide, or its derivative].

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MEANS

[Means for Solving the Problem] It traced that it was a protein-protein interaction that this invention persons have determined the important metabolic fate wholeheartedly among the metabolic fates which constitute the growth mechanism of a cancer cell as a result of examination, and since the peptide equivalent to the joint domain of the protein concerning this interaction was able to control the function of this protein, I thought that such a short peptide was useful as an anticancer agent.
[0006] Then, various oncogene products in order that the amino acid sequence of promising tripeptide may predict, For example K-Sam, Yes, Ret, Kit and Fms, ErbB, Met, Ros, Sea, Trk, Src, Fgr, Fyn, Lyn, Lck, Hck, Abl, and Arg etc. -- the consensus sequence of the oncogene product which has the array further called the Sark homology (SH) domain was searched, and it searched for the tripeptide thru/or the dipeptide which predicts those junction sequences and is equivalent to the domain. Consequently, as tripeptide, Pro-Phe-Pro, Pro-Lys-Pro, Pro-Asn-Pro, Pro-Tyr-Pro, Tyr-Asp-Pro, Tyr-Ser-Pro, Glu-Arg-Pro, Pro-Tyr-Trp, Thiopro(Thioproline)-Thr-Trp and such N-acetyl object, or C-amide object was found out, and Glu-Arg, D-Glu-Arg, and Glu-D-Arg were found out as a dipeptide. Although their eyes were turned to the drug retrieval by preventing proteinic association in the above research, it found out that some which have the activity as protease activator were in the peptide compounded to the completely unexpected thing. This invention is made based on the above knowledge.

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EXAMPLE

[Example]
Example 1 [Composition of Pro-Phe-Pro (PFP)]
Boc-Phe 2.7g (10 millimol), ProOBzl HCl 2.4g (10 millimol), and 1-hydroxy benzotriazol (HOBt) 1.5g (11 millimol) were dissolved in DMF 30ml, and WSCD HCl 1.7g (11 millimol) suspended in DMF 10ml was dropped at the bottom of ice-cooling stirring. triethylamine (TEA) -- after adding 1.8ml and checking that it is pH 7-8 with the omnipotent pH indicator paper, it stirred at the room temperature all night. After filtering and removing the precipitation which deposited and carrying out vacuum concentration of the filtrate, 300 ml ethyl acetate (EtOAc) was added and it washed in order of 10% salt (NaCl) water solution of 4% sodium hydrogencarbonate (NaHCO3), 10%NaCl water solution, 10% NaCl water solution of 0.4M citric acid, and 10%NaCl water solution. Anhydrous sodium sulfate (Na2SO4) was added to the EtOAc layer, reduced pressure distilling off of the solvent was carried out after dehydration, and reduced pressure hardening by drying of the residue was carried out in the desiccator. It is 4 Ns to this hardening-by-drying object. A hydrochloric acid/dioxane 40ml, thioanisole 4ml was added and it reacted under ice-cooling for 2 hours. Dioxane after carrying out vacuum concentration of this The actuation which adds and condenses 50ml was repeated twice, and the actuation which adds and condenses diethylether (Et2O) similarly was repeated twice. Z-Pro 1.5g (6.2 millimol) and HOBt 0.84g (6.8 millimol) were added to the hardening-by-drying object which carried out reduced pressure hardening by drying of this, and it dissolved in it at DMF 30ml. WSCD HCl 1.2g (6.8 millimol) suspended in DMF 10ml was dropped under ice-cooling stirring. After preparing to pH 7-8 by TEA 1.6ml, it was made to react at a room temperature for 1.5 hours. After making it react at 4 more degrees C all night, the precipitation which deposited was filtered and removed and vacuum concentration of the filtrate was carried out. EtOAc 300ml was added and it washed in order of 10% NaCl water solution of 4%NaHCO3, 10%NaCl water solution, 10%NaCl water solution of 0.4M citric acid, and 10%NaCl water solution. Anhydrous Na2SO4 was added to the EtOAc layer, reduced pressure distilling off of the solvent was carried out after dehydration, and reduced pressure hardening by drying of the residue was carried out in the desiccator. A hardening-by-drying object is dissolved in a small amount of methanol (MeOH), and it is sephadex. Separation purification was carried out in LH-20 column (bore [of 3cm] x die length of 55cm). It was eluted with this solvent and fractionation was carried out in 5 minutes (about 8ml) per one fraction. By TLC (Kieselgel 60F254 the system of chloroform-MeOH was used for the expansion solvent.), after the check, the fractions containing the specified substance were collected and reduced pressure hardening by drying was carried out about each fraction. This was dissolved in the mixed liquor of MeOH 20ml and a 20 ml acetic acid (AcOH), and it stirred under the nitrogen air current. It is stirring A stop and 10% After adding palladium / carbon (Pd/C) (51.60% hydrated compound) 2.0g and leading a hydrogen air current, it stirred violently by ordinary temperature ordinary pressure, and the reaction was started. After it permuted the stop in the place which absorption of hydrogen ended and nitrogen gas permuted hydrogen gas for stirring, the catalyst was carried out the ** exception. Water was added to what carried out vacuum concentration of the filtrate, and vacuum concentration was carried out once again. It is sephadex about a concentrate.

Separation purification was carried out in G-10 column (bore [of 2.6cm] x die length of 60cm). It was eluted with water and fractionation was carried out in 5 minutes (about 10ml) per one fraction. The purity of each fraction was checked by TLC (n-butanol:acetic-acid:pyridine:water =4:1:1:2 (BAPW system) was used for the expansion solvent.) (Rf value 0.39), the fractions which give the spot of the specified substance individually were collected, and reduced pressure hardening by drying was carried out. This hardening-by-drying object is dissolved in 15ml water, and it freeze-dries, and is the colorless amorphous-like specified substance. 1.0g It obtained. The overall yield so far was 28%. Elemental analysis C19H25N3O4 MW.359.43 Calculated value C 63.49, H 7.01, N 11.69. Analysis value C 59.41, H 7.42, N 10.66.

[0019] Example 2 [Composition of N-acetylation object (Ac-PFP) of Pro-Phe-Pro]

Pro-Phe-Pro 1.2g (3.3 millimol) obtained in the example 1 is dissolved in 10ml of water, and it is this. N-acetyl succinimide (N-ASI) 1.5g (10 millimol) is added, and it is 1 N. pH was stirred at the room temperature after preparation to about 7 with the potassium-hydroxide water solution all night. Sephadex after condensing reaction mixture Separation purification was carried out in G-10 column (bore [of 2.6cm] x die length of 60cm).

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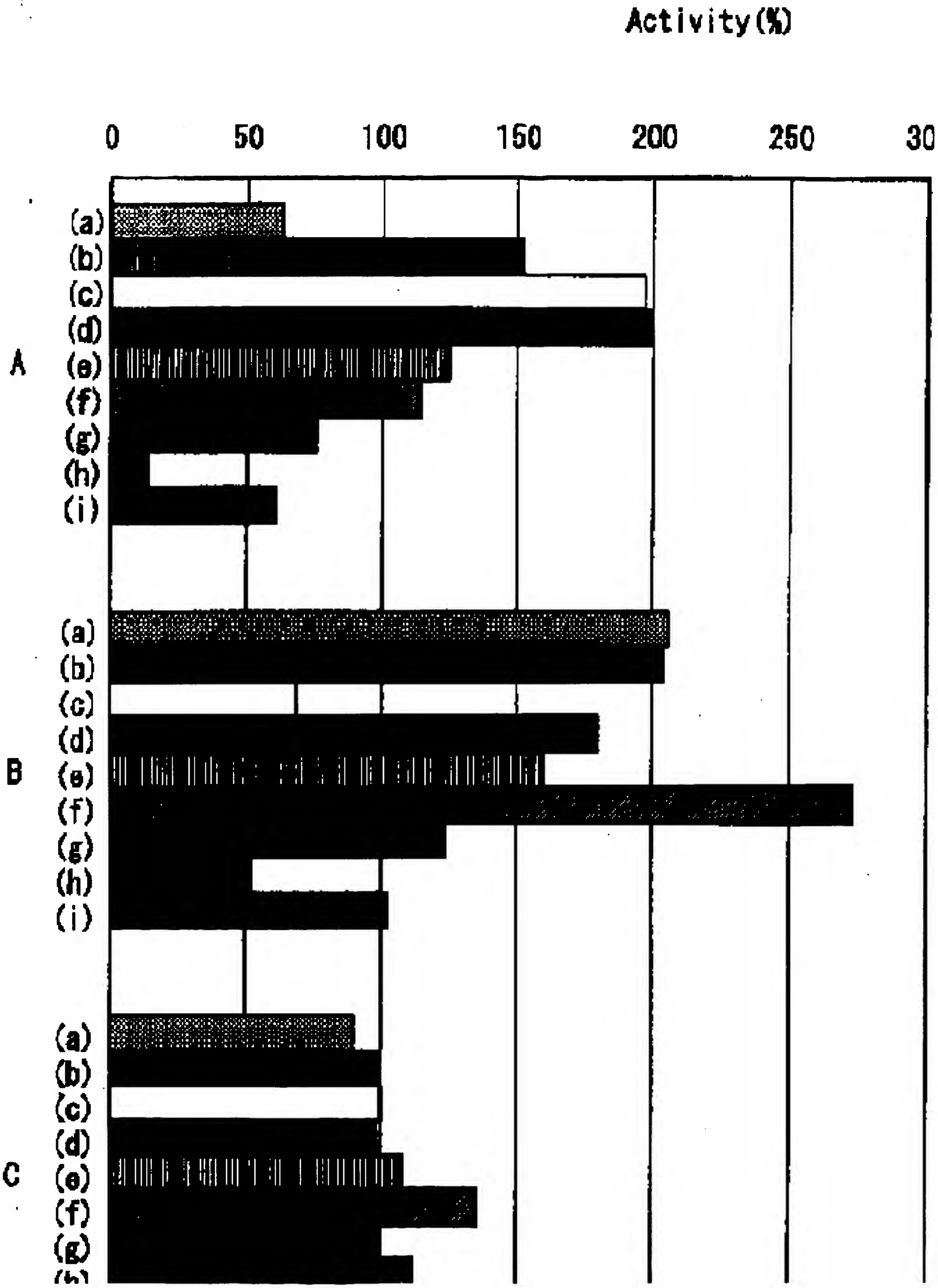
DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing showing various protease activation operations of a synthetic peptide.
[Drawing 2] It is drawing showing various protease activation operations of a synthetic peptide.
[Drawing 3] It is drawing showing various protease activation operations of a synthetic peptide.
[Drawing 4] It is drawing showing various protease activation operations of a synthetic peptide.
[Description of Notations]
A-Q in drawing shows the following peptides.
A:ThioPro-Thr-Trp
B:D-Pro-Thr-Trp
C:Glu-Arg-Pro
D: Glu-Arg-Pro-amide
E:Glu-Arg
F:D-Glu-Arg
G:Glu-D-Arg
H:Pro-Phe-Pro
I:Pro-Phe-Pro-amide
J: Pro-Thr-Trp-hydrochloride
K: Pro-Lys-Pro-amide
L:Tyr-Ser-Pro
M: N-acetyl-Pro-Phe-Pro-amide
N: Pro-Tyr-Pro-amide
O: N-acetyl-Pro-Tyr-Pro-amide
P: Pro-Tyr-Pro-sulfonate
Q: Tyr-Ser-Pro-amide

[Translation done.]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100
150
200
250
30
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(b)
(c)
(d)
(e)
(f)
(g)
(h)
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B
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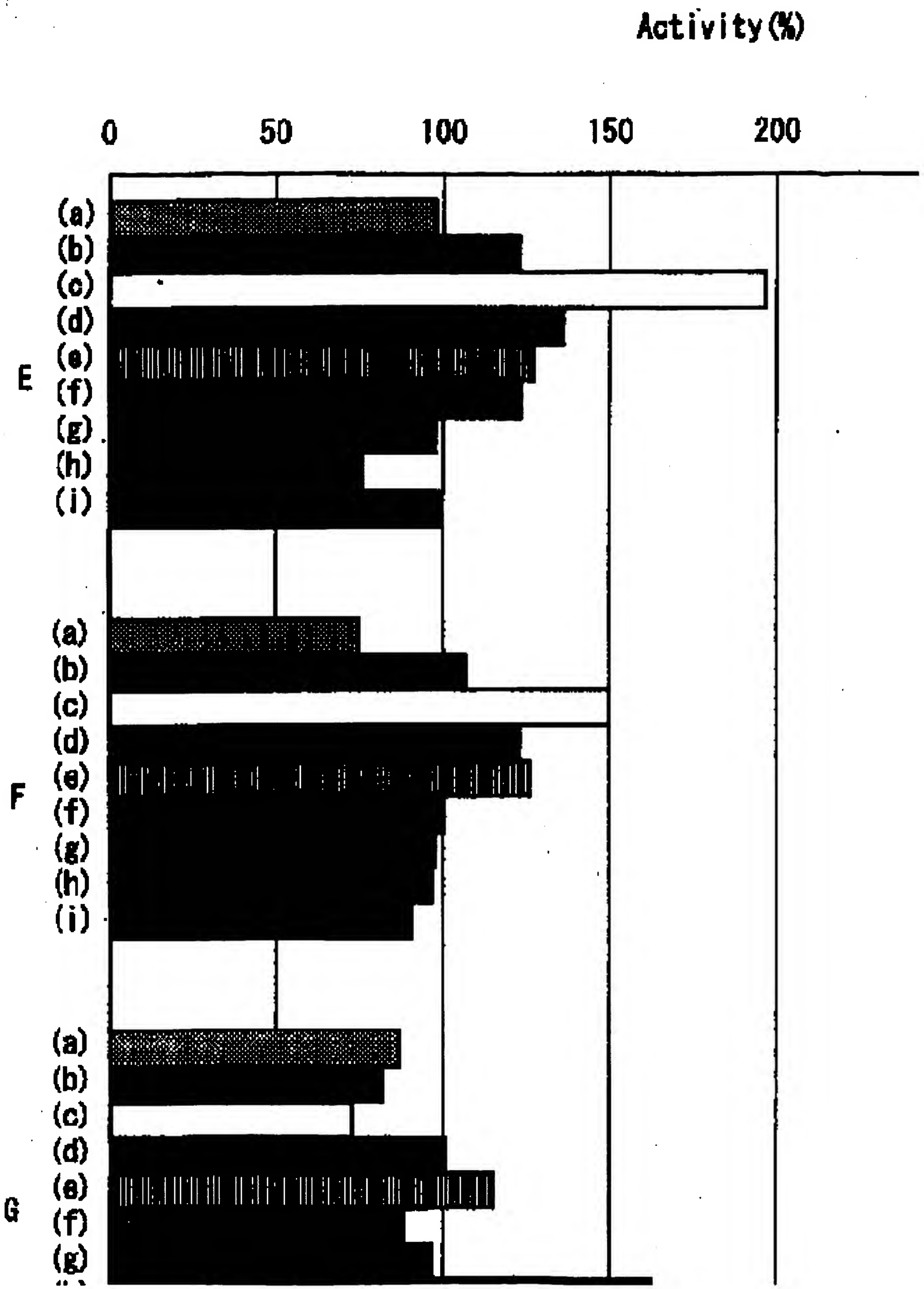
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100
150
200
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(a)
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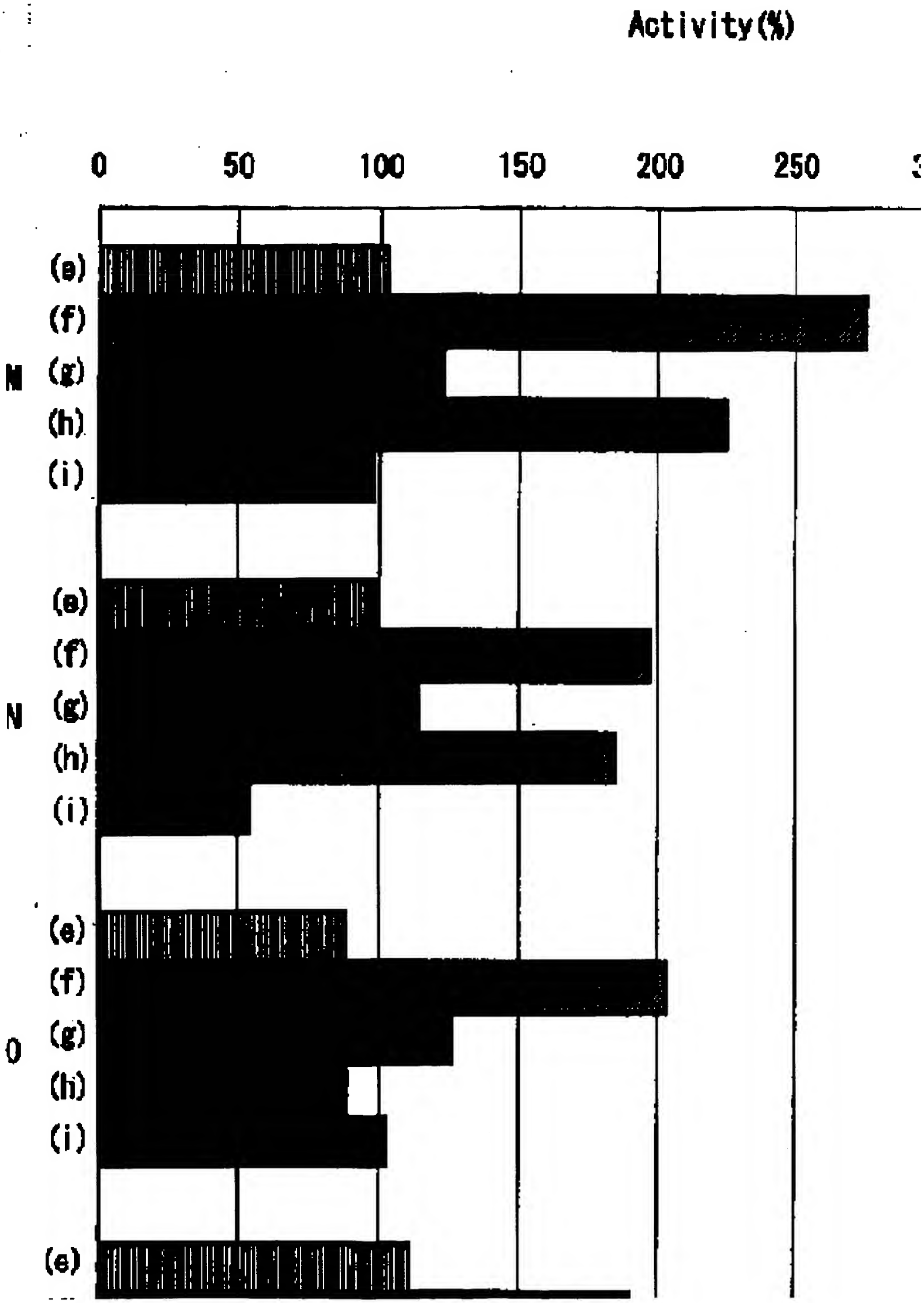
(g)
(h)
(i)


Activity(%)
0
50
100
150
200
250
(e)
(f)
(g)
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M
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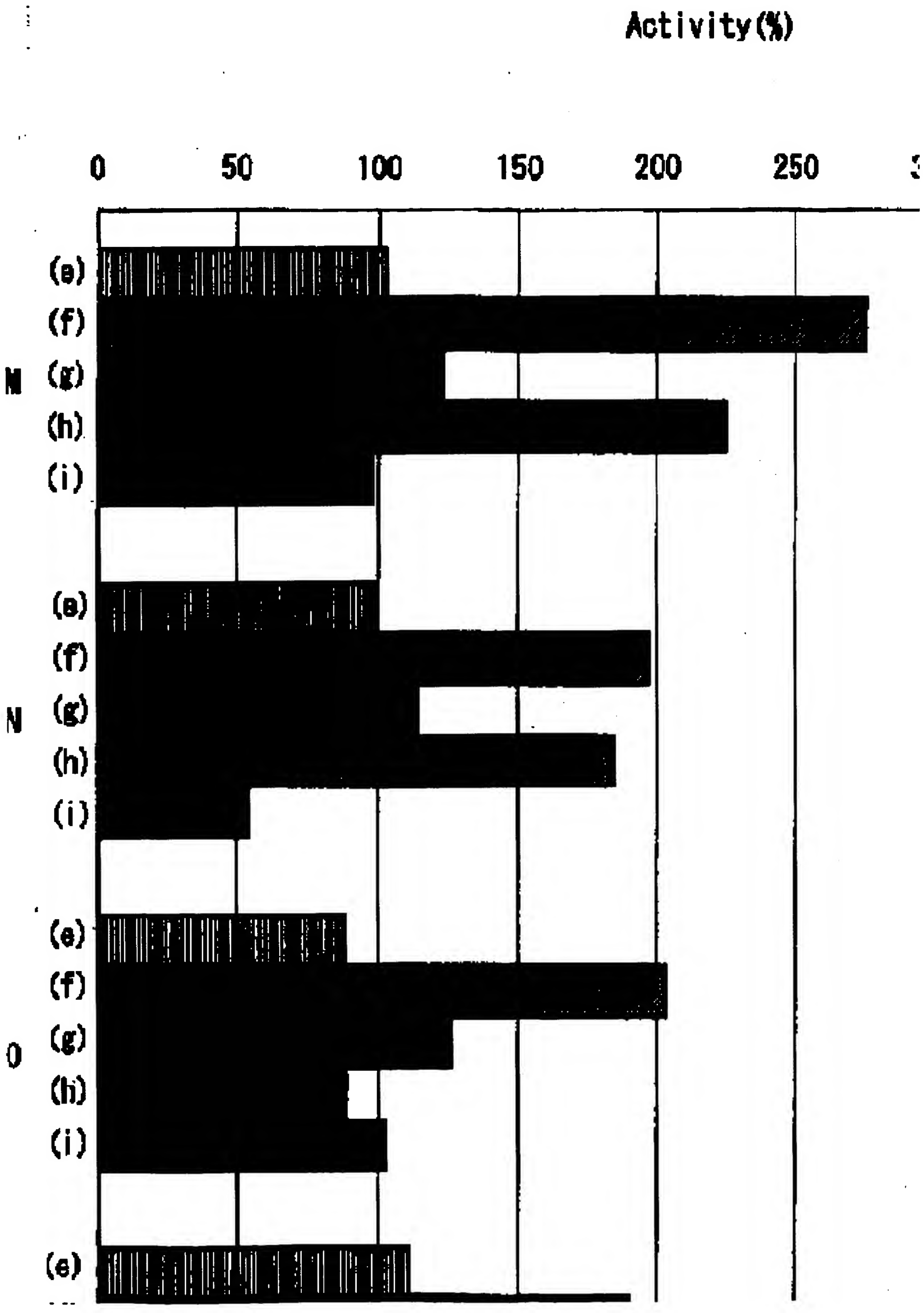
Activity(%)
0
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(e)
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